OXIDANT-ANTIOXIDANT BALANCE AND LUNG OUTCOMES: THE ROLE OF NUTRITION, GENETIC SUSCEPTIBILITY, AND CIGARETTE SMOKING

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OXIDANT-ANTIOXIDANT BALANCE AND LUNG OUTCOMES: THE ROLE OF NUTRITION, GENETIC SUSCEPTIBILITY, AND CIGARETTE SMOKING

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The aim of this research was to investigate the role of variability in antioxidant defenses in susceptibility to chronic obstructive pulmonary disease (COPD). Although exposure to cigarette smoke is the most important risk factor for COPD and is present in the vast majority of cases, only a minority of smokers develop disease. The determinants of disease susceptibility are key to understanding etiology and targeting interventions. The studies conducted for this dissertation address the hypothesis that susceptibility results from limitations in antioxidant defenses, resulting from low dietary antioxidant intake or variation in genetic factors influencing antioxidant function, to counteract the assault of oxidants present in cigarette smoke. The first project addressed the association of dietary antioxidants and rate of decline in lung function in a prospective study of elderly. Among current smokers, higher intakes of foods with antioxidant properties were each associated with an attenuated rate of decline in lung function, with the strongest associations observed in the subgroup of smokers who quit during follow-up; this study supports a role of antioxidant defenses in determining COPD susceptibility. Sequence variation in genes encoding antioxidant-related enzymes also contributes to an individual's antioxidant defenses and the second and third projects address the role of genetic variation. Given that published research on the relation of candidate genes encoding antioxidant enzymes with lung function is rapidly emerging, a systematic review of the literature

was conducted. Conclusions from this work motivated selection of candidate genes and study design decisions for the final project of this dissertation: an association study of SNPs in genes encoding antioxidant enzymes and lung function phenotypes in an elderly cohort. SNPs were selected for coverage of the full sequence variation of a broad range of antioxidant enzymes. SNPs in genes encoding members of the peroxiredoxin and isocitrate dehydrogenases were consistently associated with lung phenotypes, particularly in interaction with smoking parameters. The findings are consistent with the hypothesis that variability in the antioxidant defenses is of particular relevance in those with high oxidant burden. The results from these studies support the hypothesis that variability in antioxidant defenses contributes to COPD susceptibility.

BIOGRAPHICAL SKETCH

Amy Bentley was born in Casar, North Carolina, and enjoyed a childhood full of sunshine, creativity, and an amazing family. Although she had a broad range of interests, Amy was known as the artist in the family and was more apt to be drawing or writing than anything else. These interests continued throughout high school, leading her to study of English Literature in college.

Amy attended Guilford College in Greensboro, NC, participating in semesters abroad at the Beijing Foreign Normal College in China and at the Centro para Extranjeros at the Universidad de Guadalajara in Mexico. During her time at Guilford, Amy met and married the love of her life, Allen Bentley. She graduated with high honors with a double major in English and Spanish with a minor in East Asian Studies.

After several years working in English as a Second Language, Amy decided to formally pursue an interest in nutrition. She graduated as the top senior in her field from LaSalle University in Philadelphia, PA, with a BS in Nutrition. Amy began her studies at the Division of Nutritional Sciences at Cornell University in January, 2004 with a focus on nutritional epidemiology and chronic disease. After completing initial work on genetic risk factors associated with folate metabolism and risk of breast cancer in the Nurses' Health Study, Amy's research centered on antioxidant defenses and pulmonary function, work that would become the focus of this dissertation.

Amy was blessed with a beautiful daughter, Sydney Wren Bentley, during the course of her studies at Cornell. She plans to continue a career in research, using epidemiological principles to investigate the effects and interactions of genetic, environmental, and nutritional factors on chronic disease risk.

This work is dedicated to Allen Bentley, whose support in this and all of my efforts is the foundation upon which I stand,

and to the members of my family who have been afflicted with COPD and serve as constant reminders of the high costs associated with the lack of answers to these questions: the late Maxine Hutchins Rosser, the late John Allen Bentley, Sr., and Martha Ann Bentley.

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To my parents, Patricia and David Rosser, who firmly believe that I can do anything and share that belief at just the right times.

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LIST OF ABBREVIATIONS

ABCC1: ATP-binding cassette C1
CAT: catalase
COPD: chronic obstructive pulmonary disease
CS: current cigarette smoker
FEV_1: forced expiratory volume in the 1st second of effort
FFQ: food frequency questionnaire
FS: former cigarette smoker
FVC: forced vital capacity
G6PD: glucose-6-phosphate dehydrogenase
GCL: glutamate-cysteine ligase
GCLC: GCL catalytic subunit
GCLM: GCL modulatory subunit
GGT: gamma-glutamyl transpeptidase
GPX: glutathione peroxidase
GLRX: glutaredoxin
GSH: glutathione
GSR: glutathione reductase
GSS: glutathione synthetase
GSSG: oxidized glutathione
GST: glutathione S-transferase
Health ABC: Health, Aging, and Body Composition Study
IDH: isocitrate dehydrogenase
NS: never smoker
$PPFEV_1$: Percent achieved of predicted value for FEV_1
PRDX: peroxiredoxin
SNP: single nucleotide polymorphism
SOD: superoxide dismutase
TXN: thioredoxin
TXNRD: thioredoxin reductase

CHAPTER 1

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is characterized by the development of airflow limitation that is not fully reversible, a state that is seen in the progression of emphysema and chronic bronchitis. COPD is a major cause of morbidity(1), with symptoms including shortness of breath, decreased exercise capability, wheezing, recurrent respiratory infections, severe cough, and poor overall oxygenation. At advanced stages, COPD commonly progresses to respiratory failure and death. COPD is the fourth leading cause of mortality in the U.S.(2), and the public health burden of this disease is growing(3) .

The single most important risk factor for the development of COPD is exposure to cigarette smoke, with current or former smokers constituting approximately 80-90% of COPD patients (4, 5). Despite the clear importance of smoking in the etiology of this disease, there is considerable variation among smokers in their response to cigarette smoke exposure. Several large population-based studies reported that 17% of middle-aged smokers and 43% of older current smokers had spirometry-defined COPD(6). A landmark study of middle-aged working men found that 12% of moderate smokers and 26% of heavy smokers had airflow obstruction(7). The latter study is often cited in support of the susceptibility hypothesis, that a minority of smokers are vulnerable to the damaging effects of cigarette smoke. A dissenting view suggests the study by Fletcher et al (7) underestimates the proportion of susceptible smokers(8). Despite this controversy, the evidence is clear that pulmonary response to cigarette smoke exposure, as indicated by either spirometry or COPD diagnosis, varies substantially among smokers. Indeed, a large cross-sectional study found that only an estimated 15% of the variation in lung function was explained by smoking parameters(9).

The variability in risk of disease in cigarette smokers constitutes an unresolved and key interest in etiologic research on COPD: what distinguishes those smokers who develop airflow obstruction from those who do not? Understanding the answer to this question is an important step in understanding COPD pathogenesis and is likely to contribute to primary and secondary prevention efforts. Currently, available therapies for COPD produce only modest benefits, and most treatments do not slow the rate of decline in FEV_1(10, 11). Thus, research in this area is a high priority, particularly in light of the contribution of COPD to mortality, morbidity, and rising health care costs(1-3, 12, 13) .

The hypothesis investigated in this dissertation is that variability in antioxidant defenses contributes to the risk of COPD. This hypothesis was developed on the basis of several key observations. First, cigarette smoke is a rich source of oxidative stress, providing an estimated 10^{14} oxidants per puff(14), an immediate challenge to the antioxidant defenses in the lungs. Cigarette smokers and COPD patients have elevated levels of biomarkers of oxidative stress both in the lungs and systemically (15-20). Transient increases in oxidative stress biomarkers are also seen in COPD patients during an exacerbation, a period of greatly diminished lung function, often associated with a respiratory infection. The increased oxidative stress observed in smokers provokes inflammatory responses and the inactivation of antiproteinases; both increased inflammation and an imbalance between proteinases and antiproteinases are contributors to the pathogenesis of COPD.

Furthermore, evidence from observational studies of dietary intake of nutrients with antioxidant function, or serum antioxidant concentration, studied in relation to lung outcomes suggest that lower levels of antioxidant defense are associated with decreased lung function and higher COPD risk(21-33). Whether improving antioxidant defenses, particularly through changing nutritional status, would

ameliorate lung disease risk is a fundamental question in COPD prevention, and the research aims addressed in this dissertation were designed to provide further evidence relative to this question. Specifically, a series of studies was designed to address whether variability in antioxidant defenses contributes to the susceptibility to COPD and lower lung function, especially in current or past smokers.

The first project extended previous work on the association between dietary antioxidant intake and lung function outcomes by studying the question longitudinally in an older population comprised of both European and African Americans. Few prior studies provide evidence on lung function trajectory, although rate of change in lung function is a more informative marker of disease susceptibility than cross-sectional measurements. Indeed rate of change in FEV_1, a parameter of lung function, is an independent indicator of subsequent mortality risk at the population level. Also, prior studies were conducted on populations in middle age, and very few studies in elderly populations (and no longitudinal studies) have been conducted. This gap in the literature needed to be addressed; indeed, the highest risk group is the aging population, where both compromised antioxidant defenses and steep rates of decline in lung function co-exist. Finally, there has been no evaluation of this question in populations with a high proportion of African American seniors, although the distribution of lung function in this group is lower in comparison to European American peers. Thus, the first project investigated the relation of dietary intake of nutrients with antioxidant properties in relation to rate of change in lung function, and carefully considered whether associations were different by cigarette smoke exposure.

Although the evidence from this and previous work on the association between antioxidant nutrition and lung function is compelling, observational studies of dietary exposures are limited in causal inference. Thus, the second and third projects investigated the question with different tools by studying whether sequence variation

in genes that play a role in host antioxidant defenses are associated with lung outcomes. Several lines of evidence supported this approach, including findings of familial aggregation of COPD risk (34-37), and twin studies suggest heritability of lung function (38). Heritability estimates improved when data are limited to participants with a smoking history, suggesting a role for a gene-environment interaction.

On the basis of the fairly preliminary work in this area and to ground subsequent work, for the second project a systematic overview of the literature was completed, with a rigorous evaluation of studies on a broad range of enzymes with known antioxidant function. The review summarizes the evidence base to inform the continued investigation of antioxidant enzymes as contributors to COPD disease risk, and highlights promising results and apparent gaps to direct future work. The review considers evidence on sequence variation in genes and on differential expression of genes, as both can inform our understanding of the relation between antioxidant enzymes and disease risk.

The third project was guided by the results of the systematic review, and this project investigated sequence variation in genes encoding antioxidant enzymes in relation to lung function phenotypes in an elderly cohort. In completing the systematic review, we identified the need for a network-driven approach that carefully considers effect modification by cigarette smoking and more fully characterizes the variation in a broad range of antioxidant-related candidate genes. Thus, this project investigates the association between sequence variants across the genes and regulatory regions of an entire network of antioxidant enzymes that are expressed in the lungs in relation to lung function phenotypes of central importance in COPD risk. This association is considered in a cohort of the elderly, the Health, Aging, and Body Composition Study.

Overall, the three projects comprising the dissertation research will contribute to the understanding of the role of variability in antioxidant defenses in determining lung function outcomes (and, subsequently, COPD disease risk) upon cigarette smoke exposure. With limited treatment and prevention options (beyond smoking cessation) and the current inability to identify the smokers who belong to the "susceptible minority" with enhanced disease risk, establishing an association between antioxidant defenses and risk is an important contribution to current disease strategies and further etiological investigations. Given the anticipated increases in public health burden associated with this disease, such a contribution would be of considerable importance.

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CHAPTER 2

DIETARY ANTIOXIDANTS AND LUNG FUNCTION DECLINE: THE HEALTH AGING AND BODY COMPOSITION STUDY

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Abstract

Increased antioxidant defenses are hypothesized to decrease age- and smoking-related decline in lung function. The relation of dietary antioxidants, smoking, and forced expiratory volume in the 1st second of effort (FEV_1) was investigated in community-dwelling adults (aged 70-79 yrs at baseline) in the Health, Aging, and Body Composition Study, (Memphis, TN and Pittsburgh, PA; 1997-2007). 2,065 participants completed a food frequency questionnaire, self-reported smoking history, and had at least 2 measurements of FEV_1. In continuing smokers (reported smoking at baseline and follow-up), high vitamin C and high intake of fruits and vegetables were associated with an 18 and 29 ml/year slower rate of FEV_1 decline compared to low intake (P=0.0004 and 0.003, respectively). In quitters (reported smoking at baseline, but quit during follow-up), high intake was associated with a slower rate of decline for each nutrient studied ($p \leq 0.01$, all models): the rate of FEV_1 decline was attenuated by 56, 83, 73, and 33 ml/year in high versus low consumers of vitamins C, E, beta-carotene and fruits/vegetables, respectively. Antioxidant intake was not associated with FEV_1 decline in non-smoking participants. Increasing the intake of nutrients with antioxidant properties may attenuate lung function decline in older adults who smoke or have smoked.

Introduction

The process of aging has been described as the accumulation of oxidative damage that is incompletely repaired by the body's antioxidant defenses (1). The cause of this damage is assault by free radicals produced in the body via normal metabolic processes and inflammation, and by free radicals from the environment, including sources such as smoking and noxious gases. In the lungs, aging is associated with declining lung function, with rate of decline increasing with advancing age (2). In older adults, steeper rates of decline are associated with both an increased risk of chronic obstructive pulmonary disease (COPD) and an increased risk of all-cause mortality (2, 3). Exposure to cigarette smoke contributes to oxidative stress and accelerates the "aging" of the lung, resulting in the greater reductions in lung function observed among persons with a history of cigarette smoking (3-5).

The large surface area of the lungs is in constant contact with oxidants from the environment, but antioxidant defenses limit the damage associated with breathing oxygen. When these antioxidant defenses are overwhelmed, oxidative stress can provoke and enhance both the inflammation (6) and antiprotease inactivation (7) associated with COPD and contribute directly to tissue damage (8). Enhancing antioxidant defenses is hypothesized to reduce the accumulation of oxidative damage, thus potentially slowing the rate of decline in lung function associated with aging and smoking.

In support of this hypothesis, observational epidemiologic studies of dietary antioxidant intake, serum antioxidant concentration, and lung outcomes (spirometry at one point, rate of decline, and risk of COPD) suggest that lower levels of antioxidant defense are associated with worse outcomes (9-14) and higher COPD mortality risk (15). Although older adults are at the greatest risk of steep rates of decline in FEV_1

and would experience the greatest increases in quality of life with prevention, there is little research on the association between dietary antioxidants and FEV_1 in this age group. In addition, most studies of antioxidants and FEV_1 are cross-sectional, despite the expectation that rate of FEV_1 decline is a more informative indicator of mortality and COPD risk (2). To address the need for longitudinal evidence in older populations, we tested the hypothesis that dietary antioxidants modify the association of smoke exposure with the rate of FEV_1 decline in the cohort of older adult participants of the Health, Aging, and Body Composition study (Health ABC). Because dietary antioxidants are expected to directly protect against oxidative stress associated with *current* smoking, the primary analysis focused on the decline in FEV_1 over 4 years of follow-up with concurrent measurements of both dietary intake and smoking status. A secondary analysis investigated the hypothesis that a single measurement of antioxidant intake near study entry was associated with attenuated rate of decline during nine years of follow-up. Some of these results have been previously reported in the form of an abstract (16, 17).

Materials and Methods

The Health ABC is a prospective cohort study of 3,075 community-dwelling older adults, aged 70-79 years at baseline. High-functioning adults were recruited; specifically, participants reported themselves able to do the following without difficulty: walk ¼ mile, climb 10 steps, and perform activities of daily living. Details of this population have been previously published (18). The Health ABC study was approved by the institutional review boards of the University of Pittsburgh and the University of Tennessee; this study was approved by the Institutional Review Board for Human Participants at Cornell University.

Exclusion criteria included: missing food frequency questionnaire (FFQ) data or smoking history, < 2 spirometry tests with acceptable quality control scores (acceptable quality = no early coughs, no early termination, limited extrapolated volume, and ≤ 200 ml between two best FEV_1 values), or prevalent lung disease: spirometry-defined COPD, physician's diagnosis of COPD only, and asthma.

The 4-year follow-up analysis assessed the relation between dietary antioxidant intake data collected at the 1 year follow-up and lung function measured at study entry and 4 years later. The 9-year follow-up analysis assessed the relation between dietary antioxidant intake data collected at the 1 year follow-up and lung function data collected up to four times over a 9 year time period. This analysis included participants with at least 2 acceptable spirometry tests from any of the 4 examinations (study entry, follow-up studies at 4, 7 and 9 years).

Study variables

Spirometry was conducted during the clinical visit at study entry, and at the 4, 7, and 9 year follow-up examinations and tests were done according to standard guidelines (18).

Smoking exposure was assessed by interview at baseline and at each follow-up visit. For the 4-year follow-up analysis, smoking status was categorized as continuing smoker (report of smoking at each visit), quitter (report smoking at baseline visit but not at follow-up), former smoker (report history of smoking prior to baseline), and never smoker (report never smoking prior to baseline [smoked less than 100 cigarettes in lifetime]). For the 9-year-follow-up analysis, smoking status variables were defined as above for continuing, former, and never smokers, and a category of intermittent smoking was added to reflect participants whose smoking status changed over follow-up. Smoking parameters included pack years (average packs smoked per day multiplied by number of years smoking) and current smoking status at each follow-up

examination.

Dietary antioxidant intake was determined by a modified Block FFQ administered at the 12 month examination (details previously published (19)), which captures usual diet in the preceding 12 month period. The intake of nutrients with antioxidant properties was measured by whole food intake (fruits and vegetables, servings per day) and calculated micronutrients (vitamins C and E, and β-carotene). Due to limitations in data regarding dose and contents of supplements/multivitamins, analysis of antioxidant intake from these sources was not conducted. Power terms and categorical variables were created to determine if continuous variables had a linear relation with the outcome; we found no evidence of non-linearity and continuous coding was used for each of the nutrient intake variables. Product terms were created to test interaction between measures of oxidative stress (smoking) and antioxidant status (dietary antioxidant intake).

Data analysis

Linear mixed models (Proc MIXED, SAS) were used to assess the association of predictors (dietary antioxidant intake and smoking parameters) with FEV_1. Age, gender, height, race, Health ABC study site (Memphis, Tennessee or Pittsburgh, Pennsylvania) were included as covariates in all models. Rate of decline was calculated as the difference between FEV_1 measurements at each time point divided by the time between the measurements, and association between predictors and rate of decline was determined by evaluating interaction terms between the predictor and a time variable.

A variable quantifying the amount of time elapsed from study entry to each subsequent FEV_1 measurement was included in all of the models; in the 9-year follow-up analysis, the square and cube of the elapsed time variable were highly statistically significant ($p<0.0001$) and were also included.

Fruit and vegetable intake were considered as a single variable defined as the sum of the intake of fruits and vegetables (servings per day). Results of each of the models were further evaluated by comparing predicted FEV_1 values for high and low intake (high and low intake defined as the 90^{th} and the 10^{th} percentile, respectively, of the cohort distribution of intake for each of the dietary variables). Predicted FEV_1 values were calculated using model parameters and estimated within the mixed procedure of SAS.

All analyses were conducted using SAS v 9.1 (SAS, Cary, NC).

Results

Of the 2,216 participants with dietary and smoking data and without prevalent lung disease (72% of all Health ABC participants), 1,443 had valid spirometry at baseline and after 4 years of follow-up (65%, included in the 4-year follow-up analysis), and 1,720 had at least 2 valid spirometry tests over 9 years of follow-up (78%, included in the 9-year follow-up analysis); 774 participants had valid spirometry at all four time points. The proportion of eligible (i.e., still alive) participants who did not have spirometry data increased over the follow-up period: 7%, 19%, 27% and 29% were missing, respectively, at the 4 successive examinations. Among participants who completed spirometry, about 94% of tests met acceptability criteria at each examination.

Baseline characteristics of participants included in either of the analyses or excluded from the study are given (Table 2.1). Inclusion was associated with survivorship and, as expected, participants in the longitudinal analyses were more likely to be nonsmokers and, if they were smokers, to have smoked less on average over their lifetime.

Table 2.1: Baseline Characteristics of Health, Aging, and Body Composition Participants Included and Excluded from Analyses; Memphis, TN and Pittsburgh, PA; 1997-2007[a].

	4-Year Follow-Up (n=1443)	9-Year Follow-Up Additional Participants[b] (n=277)	Excluded (n=1355)
Age, years	73.5 (2.8)	73.5 (2.9)	73.8 (2.9)
Females (%)	731 (51%)	162 (58%)	691 (51%)
Blacks (%)	505 (35%)	105 (38%)	671 (50%)
Memphis, TN site (%)[c]	652 (45%)	155 (56%)	741 (55%)
Former Smokers (%)	640 (44%)	128 (46%)	636 (47%)
Current Smokers (%)	97 (7%)	18 (7%)	203 (15%)
Packyears	30.3 (27.7)	29.7 (26.9)	38.6 (31.4)
Dietary Intake			
Vitamin C, mg/day	146 (76)	143 (76)	146 (81)
Vitamin E, α-TE/day	11.1 (6.9)	10.9 (6.1)	11.0 (6.9)
β-carotene, µg/day	3392 (2532)	3534 (3036)	3631 (3199)
Fruits, servings/day	2.0 (1.2)	2.0 (1.1)	1.9 (1.2)
Vegetables, servings/day	3.0 (1.9)	3.0 (1.9)	2.9 (1.9)
FEV_1 at baseline, ml[d]	2326 (601)	2209 (600)	1945 (632)

Abbreviations: α-Tocopherol Equivalents (α-TE); Forced Expiratory Volume in the 1st second of effort (FEV_1); Chronic Obstructive Pulmonary Disease (COPD).

[a] Unless otherwise indicated, mean (SD) is reported.

[b] Characteristics of participants who were not included in the 4-year analysis, but who met inclusion criteria for the 9-year analysis (all participants in the 4-year follow-up column were included in the 9-year analysis, n=1720 total).

[c] vs. Pittsburgh, PA site

[d] Calculated on those with an acceptable baseline FEV_1 (4-year follow-up: n=1443; 9-year follow-up additional participants: n=162; Excluded: n=1098)

Rate of decline by smoking status

Mean baseline FEV_1 (adjusted for age, race, gender, height, Health ABC site, pulmonary drug use, pack years) in never smokers [2.32 L (95% confidence interval (CI) 2.29, 2.35)] and former smokers [2.33L (95% CI 2.30, 2.36)] was about 150 ml higher than mean baseline FEV_1 in continuing smokers [2.19 L (95% CI 2.07, 2.30)] and intermittent smokers [2.15 ml (95% CI 2.05, 2.25)]. Cigarette smoking was associated with a higher rate of decline in FEV_1 (P=0.05; Figure 2.1); the annual rate of decline in FEV_1 was 50 ml/yr, 52 ml/yr, 60 ml/yr and 43 ml/yr in never, former, continuing, and intermittent smokers, respectively.

Antioxidant intake

The association between antioxidant intake and lung function was evaluated using both calculated micronutrient variables (dietary vitamin C, vitamin E, and β-carotene) and intake of whole foods with antioxidant properties (fruits and vegetables). Although dietary intake was represented with continuous variables, results are presented as high (90^{th} percentile) and low (10^{th} percentile) levels for convenience. Participants with the highest burden of environmental oxidative stress were assumed to be continuing smokers and quitters. In these groups, high antioxidant intake was consistently associated with a slower rate of decline (Table 2.2), but there was little or no association of dietary variables to lung function decline in never and former smokers.

Continuing smokers who consumed diets high in either vitamin C or vitamin E had slower rates of decline in FEV_1 (-30 and -32 ml/year) compared to *continuing smokers* who consumed diets *low* in these nutrients (-48 and -47 ml/yr; P interaction = 0.0004). A more dramatic difference was observed between continuing smokers with high vs. low fruit and vegetable intake, with a 29 ml/year attenuation in rate of decline

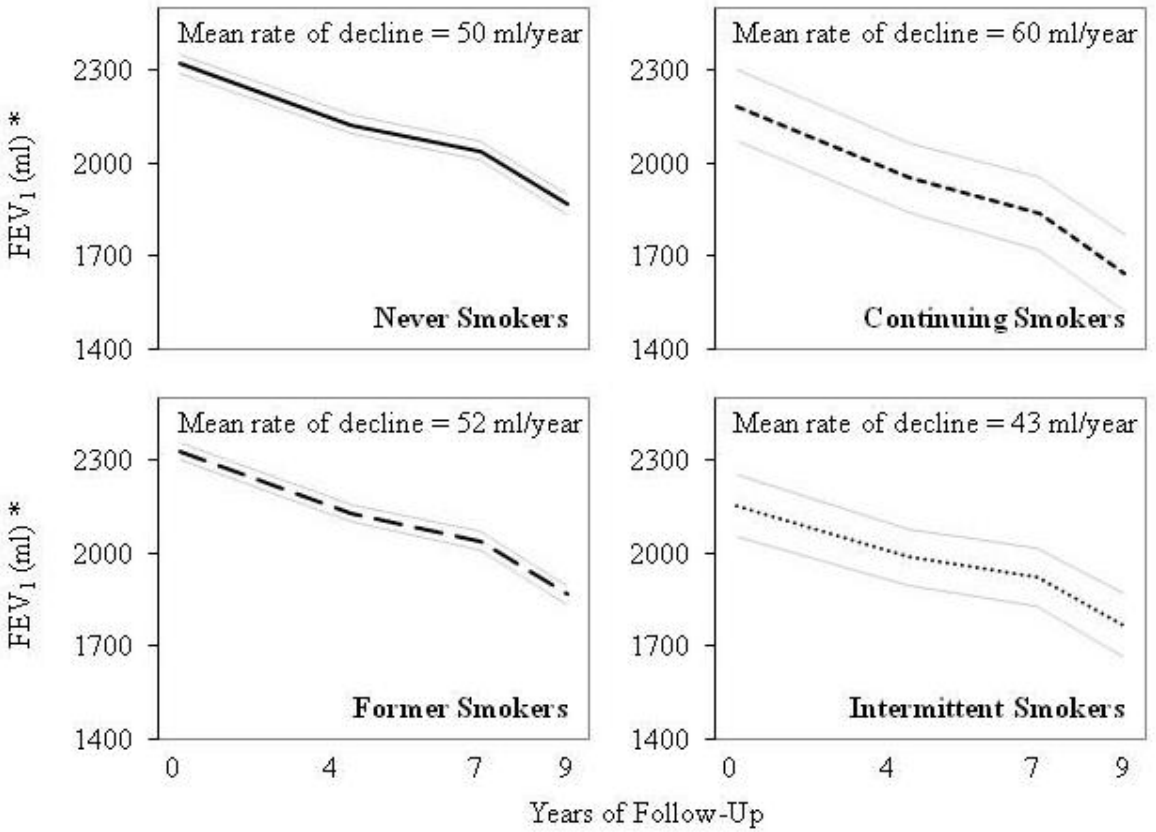

Figure 2.1: Rate of Decline in FEV_1 by Smoking Status: the Health, Aging, and Body Composition cohort (1997-2007); Memphis, TN and Pittsburgh, PA.

——— Never Smokers (n=835); — — Former Smokers (n=758); - - - Continuing Smokers (n=55); ····· Intermittent Smokers (n=72); ——— 95% CIs

**FEV_1 adjusted for age, race, gender, height, packyears, Health ABC site, and use of pulmonary drugs.*

Table 2.2 Difference in Rate of Decline in FEV_1 for High (90th %) vs. Low (10th %) Dietary Antioxidant Intake[a]

Nutrient	Smoking Status	High Intake (90th percentile) Baseline FEV_1	High Intake (90th percentile) 4th year FEV_1	High Intake (90th percentile) FEV_1 Decline	Low Intake (10th percentile) Baseline FEV_1	Low Intake (10th percentile) 4th year FEV_1	Low Intake (10th percentile) FEV_1 Decline	High vs. Low Rate of Decline[b,c,d]
Vitamin C	Continuing Smoker	2129 ml	2009 ml	-30 ml/year	2117 ml	1923 ml	-48 ml/year	+18 ml/year
236.5 vs 65.2 mg/day	Quitter[e]	2174 ml	2181 ml	+2 ml/year	2140 ml	1922 ml	-54 ml/year	+56 ml/year
	Former Smoker	2334 ml	2097 ml	-59 ml/year	2310 ml	2123 ml	-47 ml/year	-12 ml/year
	Never Smoker	2380 ml	2174 ml	-51 ml/year	2339 ml	2156 ml	-46 ml/year	*No difference* ($P = 0.0004$)
Vitamin E	Continuing Smoker	2070 ml	1943 ml	-32 ml/year	2172 ml	1982 ml	-47 ml/year	+16 ml/year
19.5 vs 5.1 α-TE/day	Quitter[e]	2124 ml	2200 ml	+19 ml/year	2202 ml	1947 ml	-64 ml/year	+83 ml/year
	Former Smoker	2295 ml	2077 ml	-54 ml/year	2339 ml	2135 ml	-51 ml/year	*No difference*
	Never Smoker	2386 ml	2191 ml	-49 ml/year	2343 ml	2148 ml	-49 ml/year	*No difference* ($P = 0.002$)
β-carotene	Continuing Smoker	2092 ml	1926 ml	-42 ml/year	2167 ml	2003 ml	-41 ml/year	*No difference*
6592 vs 1186 μg/day	Quitter[e]	2130 ml	2211 ml	+20 ml/year	2188 ml	1977 ml	-53 ml/year	+73 ml/year
	Former Smoker	2301 ml	2089 ml	-53 ml/year	2331 ml	2122 ml	-52 ml/year	*No difference*
	Never Smoker	2362 ml	2159 ml	-51 ml/year	2354 ml	2169 ml	-46 ml/year	*No difference* ($P = 0.01$)
Fruits and	Continuing Smoker	2092 ml	1994 ml	-24 ml/year	2184 ml	1972 ml	-53 ml/year	+29 ml/year
Vegetables	Quitter[e]	2104 ml	2038 ml	-17 ml/year	2251 ml	2053 ml	-50 ml/year	+33 ml/year
8 vs 2 servings/day	Former Smoker	2341 ml	2105 ml	-59 ml/year	2300 ml	2113 ml	-47 ml/year	-12 ml/year
	Never Smoker	2375 ml	2178 ml	-49 ml/year	2337 ml	2147 ml	-48 ml/year	*No difference* ($P = 0.003$)

Abbreviations: α-TE, α-Tocopherol Equivalents; Forced Expiratory Volume in the 1st second of effort (FEV_1);

[a] Rate of decline estimated from hierarchical mixed models adjusted for age, height, race, gender, pulmonary drug use, study site, current cigarettes/day, and calorie intake (separate models for each listed antioxidant).

[b] **Positive** (+) differences indicate an attenuated rate of decline in participants with higher dietary intake of nutrient considered.

[c] "No Difference" indicates difference between groups less than 10 ml/year.

[d] *P* value given is for the interaction term for smoking status, dietary antioxidant intake, and time.

[e] Estimates in this category correspond to those who quit smoking during the study follow-up.

(*P* interaction = 0.003). No difference was observed among continuing smokers by intake of β-carotene.

In the group of smokers who quit, high antioxidant intake (vs. low) was associated with greater attenuations in rate of decline, and the effects were greater than those observed in continuing smokers. High vs. low intake was associated with a 56, 83, and 73 ml/year slower decline for vitamins C, E, and β-carotene, respectively (*P*s for interaction 0.0004, 0.002, and 0.01). These differences reflect *increases* in pulmonary function over the 4 years of follow-up among quitters with higher antioxidant intake of these nutrients compared with the declines observed among quitters with low antioxidant intake. Among smokers who quit, a high intake of fruits and vegetables (vs. low) was also associated with an attenuated rate of decline (33 ml/year), and the magnitude of this attenuation was similar to that observed in continuing smokers (29 ml/year attenuation in rate of decline).

When dietary vitamin C, vitamin E, and β-carotene were included in the same model to account for multicollinearity, a high intake of all three antioxidants (compared to low intake of all) was associated with a 20 ml/year slower decline among continuing smokers and a 94 ml/year slower decline among quitters. The statistical significance of all terms was reduced, and the interaction between vitamin C, smoking status, and time had the lowest p-value (*P*=0.08).

When the analysis was extended to include the follow-up data on FEV_1 over the nine year time period, all associations were attenuated. In continuing smokers consuming a diet high in antioxidants, models incorporating all available lung function data yielded effects of similar magnitude: in continuing smokers high vitamin C was associated with an 18 ml/year slower decline; high vitamin E was associated with an 18 ml/year slower decline; and high fruit and vegetable intake was associated with a 14 ml/year slower decline. No associations were observed among intermittent,

former, or never smokers in the 9-year follow-up analysis. No associations were observed with dietary intake of β-carotene in the 9-year follow-up analysis.

The 9-year analysis was limited to the 1,443 participants included in the 4-year analysis to determine if the difference in results was due to selection bias instead of the increased time of follow-up. This analysis yielded larger associations with increased statistical significance (though still not the same level of statistical significance as in the 4-year analysis): in continuing smokers, high vitamin C was associated with an 23 ml/year slower decline (P=0.06); high vitamin E was associated with a 27 ml/year slower decline (P=0.20); and high fruit and vegetable intake was associated with 21 ml/year slower decline (P=0.32). No associations were observed among intermittent, former, or never smokers, and no associations were observed with dietary intake of β-carotene.

Discussion

Dietary antioxidant intake is a potentially modifiable predictor of the decline in FEV_1 in this longitudinal study of well-functioning older adults. Among the Health ABC participants, consuming a diet high in antioxidants was associated with an attenuated slope of decline in lung function in current smokers, the population subgroup at greatest risk of lung-related disability and death. In smokers, higher intakes of vitamin C, vitamin E, β-carotene, and fruits and vegetables were associated with meaningful differences in the trajectory of change in FEV_1. In Health ABC cohort participants who continued to smoke over four years of follow-up, Vitamin C and fruit and vegetable intake had the strongest associations; smokers with *high* intakes had 18 and 28 ml/year *lower* rate of decline in FEV_1 compared to smokers with low intakes. In participants who quit smoking, all dietary antioxidants had strong

inverse associations with rate of decline: quitters with *high* intakes of vitamin E, β-carotene, vitamin C and fruit and vegetables had 83, 73, 52 and 32 ml/year *lower* rate of decline in FEV_1, respectively, compared to quitters with low intake. Although the association of fruits and vegetables was similar in continuing smokers and quitters, suggesting a broad role for these foods in persons who are smoke-exposed, the stronger findings for vitamins C, E, and β-carotene in quitters may signify the importance of a range of antioxidants to optimally support the transition from current to former smoker.

Prior studies of younger adult populations reported associations between dietary antioxidant intake and rate of decline in FEV_1 similar in magnitude to the Health ABC findings. Changes over time in the consumption of fresh fruit were associated with rate of decline in FEV_1 in healthy British adults (age 18-73): participants with the largest *decrease* in fruit intake had a *steeper* rate of decline in FEV_1 (89 ml/year and 133 ml/year steeper for men and women) compared to participants with no change in fruit intake (20), and effects were strongest among cigarette smokers. This study also reported that average fruit intake had little or no association with rate of decline, underscoring the importance of appropriately timed measurements of dietary intake. In middle-aged Welshmen (age 45-59) followed for 5 years, the rate of FEV_1 decline was 10 – 15 ml/year *slower* in men with *high* apple intake compared to men with low intake; however, no associations were found for vitamins C, E, and β-carotene (9). Nottingham adults (age 18-70) consuming diets *high* in vitamin C (100 mg higher than low vitamin C diets) had an *attenuation* in the rate of decline in FEV_1 (by about 6 ml/yr), but no associations were found for vitamins A and E (14). In a cohort of heavy smoking French adults (age 20-44), *higher* levels of serum β-carotene and serum vitamin E were associated with *slower* rates of decline in FEV_1 (21).

Despite the relatively few longitudinal studies of dietary antioxidants and lung function, most of the cross-sectional studies that have investigated this hypothesis reported positive associations between vitamins C and E, β-carotene, fruits and vegetables and lung outcomes (9-15, 20-26). On closer examination, the strongest effect sizes were often identified in cigarette smokers.

Among the Health ABC participants classified as never and former smokers, the intake of nutrients and foods with antioxidant properties had little or no association with the rate of decline in FEV_1. These findings are consistent with the hypothesis that a protective effect of nutrients with antioxidant properties is expected primarily in persons with a high burden of oxidative stress. Thus, the role of dietary antioxidants may be to provide additional functional capacity to reduce damage from exposure to exogenous oxidants. The findings suggest a testable hypothesis, which could be pursued in other studies: non-smokers may not benefit from increasing the intake of nutrients with antioxidant properties either because their overall level of oxidant burden is low enough to be well-managed by endogenous capacity, or because the dietary antioxidants considered in Health ABC are not efficacious at bolstering *endogenous* antioxidant capacity.

The Health ABC study allows a closer look at the rate of decline in lung function associated with smoking status in a cohort of older adults. Of particular interest is the difference in rate of decline in FEV_1 between continuing smokers (60 ml/year) and intermittent smokers (43 ml/year). These data suggest that at advanced ages significant functional improvements may be attained with smoking cessation (even with relapses). Attenuating the rate of decline is especially important in the subgroup of smokers, because, on average, smokers have lower starting FEV_1 values (150 ml lower), and are therefore at greater risk of disability and death.

The findings in Health ABC underscore the importance of timing of exposure

measurements relative to outcome. If the balance between antioxidants and oxidants in the lung compartment is expected to affect the rate of decline in lung function, then *concurrent* measurements of diet, smoking parameters, and lung function are likely to be most informative. In the Health ABC study, the association between antioxidant intake and rate of decline in smokers is strongest (effect size and statistical significance) when the analysis is limited to the time period with concurrent measurements. When the analysis is extended to investigate the relation of baseline diet to longer-term effects on rate of decline in FEV_1, the magnitude of the associations were similar, but less statistically significant. Including endpoint measurements separated in time from exposure measurements may introduce "noise" in the analysis. In the Health ABC study, the dietary intake of antioxidants was estimated from a single food frequency questionnaire administered in the first 12 months of the study. In this vulnerable age group, many factors are associated with changes in dietary quality including new disease diagnoses, the onset of functional impairment, changes in living situation, loss of a spouse, etc.(27-29). Thus, the strongest analytic approach is arguably one that limits consideration of pulmonary function changes to the same time period in which diet was measured.

Our study has several strengths. The study used data from a large, prospective cohort study of elderly people; a population which stands to benefit from research aimed at understanding how to prevent loss of lung function. The quality control standards for spirometry in Health ABC followed best practice, as evidenced by the excellent overall quality of the pulmonary function data. The use of two separate methods to estimate dietary intake of nutrients with antioxidant properties, micronutrients and whole foods, yielded similar results confirming our assumption that the findings represent true associations of diet on lung function. The consideration of simultaneous models including all nutrients diminishes the possibility that the

observed associations were primarily driven by a single dietary constituent.

There are also several limitations of the study. While the use of antioxidant supplements is expected to contribute to overall antioxidant status, limitations in the Health ABC data collection methods for supplements precluded their further consideration. A study conducted in an elderly cohort raises a concern about survivor bias: non-random loss-to-follow-up results in healthier participants at later stages of the study. Survivor bias is evident when participants contributing data at each successive measurement are compared: participants who were followed for longer were younger, more often female, and smoked less at baseline. If participants who did not remain in the study had a greater burden of oxidative stress (given their smoking history and age, this may be a reasonable hypothesis) then, in light of the findings in participants with the highest burden of oxidative stress, even stronger associations than those observed might be expected.

In summary, this study of older adults (70-79 years at baseline) supports the hypothesis that nutrients with antioxidant properties are associated with a slower rate of FEV_1 decline in current and quitting smokers. Whether changing the intake of foods rich in nutrients with antioxidant properties will lead to a slower rate of decline in a FEV_1 remains to be demonstrated, but the results from the Health ABC cohort support this inference.

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CHAPTER 3

GENETIC VARIATION AND GENE EXPRESSION IN ANTIOXIDANT-RELATED ENZYMES AND RISK OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A SYSTEMATIC REVIEW

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Abstract

Observational epidemiologic studies of dietary antioxidant intake, serum antioxidant concentration, and lung outcomes suggest that lower levels of antioxidant defenses are associated with decreased lung function. Another approach to understanding the role of oxidant/antioxidant imbalance in risk of Chronic Obstructive Pulmonary Disease (COPD) is to investigate the role of genetic variation in antioxidant enzymes, and indeed family-based studies suggest a heritable component to lung disease. Many studies of the genes encoding antioxidant enzymes have considered COPD or COPD-related outcomes, and a systematic review is needed to summarise the evidence to date, and to provide insights for further research.

Genetic association studies of antioxidant enzymes and COPD/COPD-related traits, and comparative gene expression studies with disease or smoking as the exposure were systematically identified and reviewed. Antioxidant enzymes considered included enzymes involved in glutathione (GSH) metabolism, in the thioredoxin (TXN) system, superoxide dismutases (SOD), and catalase (CAT).

A total of 29 genetic association and 14 comparative gene expression studies met the inclusion criteria. The strongest and most consistent effects were in the genes *GCL*, *GSTM1*, *GSTP1*, and *SOD3*. This review also highlights the lack of studies for

genes of interest, particularly *GSR*, *GGT*, and those related to *TXN*. There were limited opportunities to evaluate a gene's contribution to disease risk through a synthesis of results from different study designs, as the majority of studies considered either association of sequence variants with disease or effect of disease on gene expression. Network-driven approaches that consider potential interaction between genes and among genes, smoke exposure, and antioxidant intake are needed to fully characterise the role of oxidant/antioxidant balance in pathogenesis.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is characterised by the development of airflow limitation that is not fully reversible. COPD is a major, and growing, public health burden (1).

Smoking is the most important risk factor for COPD; however, there is considerable variation in the response to smoke exposure (2), and it has been estimated that only 15% of the variation in lung function is explained by smoking parameters (3). While not discounting the paramount importance of smoke exposure in the development of COPD, clearly other factors are significant. Genetic variation is a prime candidate, and many recent studies explore the contribution of genetic variation to inter-individual differences in the response to cigarette smoke.

This review focuses on genes related to antioxidant activity, as oxidant-rich cigarette smoke strains the antioxidant defenses of the lungs, leading to direct tissue damage and contributing to the inflammation and antiprotease inactivation seen in COPD. This hypothesis is supported by epidemiologic studies associating low dietary antioxidant intake and serum antioxidant concentration with decreased lung function (4-9) and increased COPD mortality risk (10).

Many genetic association studies and comparative expression studies

investigate the relation between genes coding for antioxidant enzymes and either COPD or associated traits. An overview of the evidence is warranted to ascertain whether the pattern of published results suggests directions for future research, or whether there are apparent gaps that need to be addressed. Both genetic association studies and gene expression studies were included: polymorphisms can affect disease risk in ways that may or may not be mediated by changes in expression, and expression studies can provide a snapshot of the adaptive response to an exposure. Thus, we conducted a systematic review of the literature to characterise the evidence that genes coding for antioxidant enzymes contribute to the aetiology of COPD and related traits.

Methods

The selection of genes was based mainly on delineating important proteins and the networks of genes that may influence the amount or function of those proteins (Figure 3.1). As glutathione (GSH) is an antioxidant that plays a significant role in the lung, genes encoding GSH-associated enzymes were selected. Thioredoxin, which reduces oxidized glutathione and has an antioxidant function that overlaps GSH function, was included with genes encoding associated enzymes. Catalase and superoxide dismutase, two classical antioxidant enzymes of the lung, were also selected. Searches of PubMed were performed up to August 2007 (further details in Supplement A.1). Published papers considering gene-disease association or differential gene expression in adult humans were selected. Association studies were included if the outcome was disease or lung function. Expression studies were included if the experimental exposure was disease status or smoking and if expression was measured in pulmonary tissues or cells.

Results

A total of 29 genetic association studies and 14 expression studies were identified (Table A.2). The results of these studies are presented in supplemental tables (Association Studies, Table A.3; Expression Studies, Table A.4) and summarized below.

Glutathione Synthesis

Three enzymes that relate to glutathione synthesis were considered: gamma-glutamyl transpeptidase (GGT, no studies found), glutamate-cysteine ligase (GCL), and glutathione synthetase (GSS). A substitution in the promoter region of *GCLM* (GCL's

modulatory subunit), leading to decreased glutathione levels (11), was associated with a 3-fold increased risk of COPD in Chinese smokers (12). In the single study of a substitution in GCL's catalytic subunit (GCLC) that results in decreased expression (13), an increased prevalence was observed in patients vs. healthy controls (OR 1.83, 95% CI[1.00, 3.36]) (14).

Expression studies of glutathione synthesis compared expression in COPD patients with asymptomatic smokers and/or nonsmokers. Eight of the 9 comparisons of *GCLC* expression in lung epithelium found upregulation with disease (15-18). Two comparisons of *GCLM* expression in lung epithelium of lung tumor patients with and without COPD showed decreased *GCLM* expression with disease (17), while the single study of COPD patients without lung tumor found upregulation (18).

There were 4 comparisons of *GCL* expression in lung epithelium of asymptomatic smokers and nonsmokers: expression of both subunits was increased in smokers who were healthy volunteers (18, 19) and unchanged or decreased in smokers with lung tumor (17).

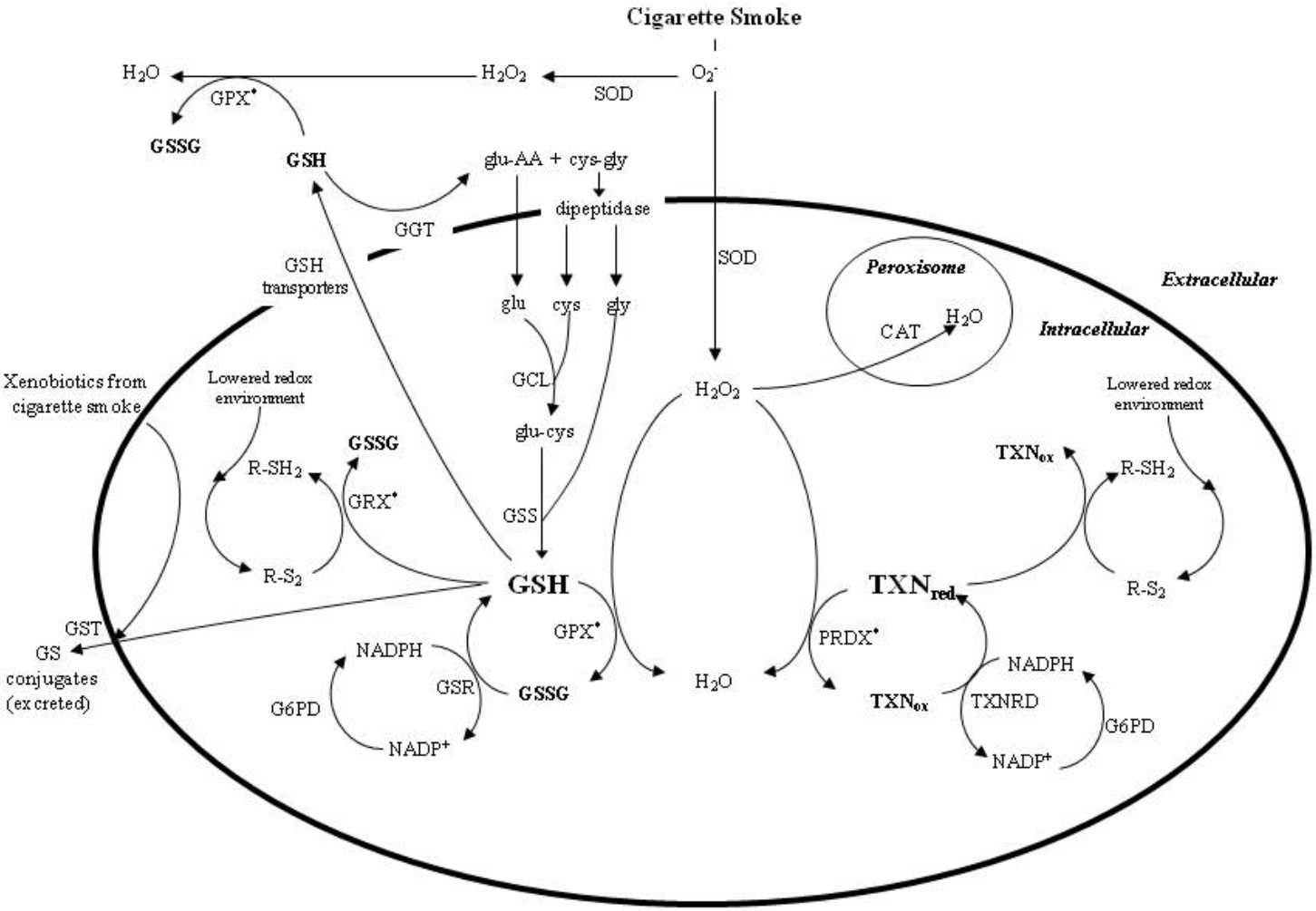

FIGURE 3.1 Interaction of antioxidant enzymes in response to oxidative stress
**this reaction also occurs without the listed enzyme*

Expression of the *GCL* subunits in alveolar macrophages/inflammatory cells showed a more consistent pattern. Both *GCLC* (16, 17, 20) and *GCLM* (20) were increased in COPD patients vs. smokers, and smokers had lower expression of both subunits compared to nonsmokers (17, 20). A 27% upregulation of the mRNA of *GSS*, the final step of GSH synthesis, was reported in smokers vs. nonsmokers (p=0.08) (19), but was not replicated in comparisons of nonsmokers with either asymptomatic smokers or COPD patients (18, 19).

Antioxidant Activity of GSH and Recycling

Glutathione peroxidase (GPX), glutaredoxin (GLRX), glutathione reductase (GSR), and glucose-6-phosphate dehydrogenase (G6PD) were considered for their role in the antioxidant activity of GSH and in GSH recycling. There were no association studies of variants in these genes.

Two studies evaluated expression in COPD patients versus healthy controls. In the single study of *GPX2*, it was strongly upregulated in COPD patients at all stages compared to nonsmokers (and modestly upregulated compared to smokers) (18). *GPX3* was upregulated in COPD patients compared to nonsmokers, though this difference was not seen in comparison with asymptomatic smokers (18, 21). There was little evidence of differential regulation of *GPX4*, *GPX5*, or *GPX7* by disease status (18).

GPX2 showed a 3 to 5-fold upregulation in epithelial cells of smokers compared to nonsmokers.(18, 19) Each of the 4 studies of *GPX3* expression in epithelial cells reported upregulation in smokers: two studies found a 2-fold difference (19, 22), and a study of alveolar macrophages reported similar differences (22).

Two expression studies evaluated regulation of *GLRX*. A statistically significant downregulation was observed in the tissue homogenate of COPD patients

compared to smokers (patients had either resection for lung tumor or lung transplantation for severe COPD) (23), but similar results were not observed in an analysis of bronchial epithelial cells (18). A statistically significant upregulation of GLRX in the sputum of COPD patients in exacerbation was reported vs. nonsmokers (23).

The sole comparison of GSR expression in epithelial cells by disease groups showed upregulation in COPD patients (18). In the 2 comparisons by smoking status, upregulation was observed among smokers (18, 19). Very similar results were found in the 3 studies of *G6PD* expression: 2-fold upregulation in epithelial cells (18, 19) and in alveolar macrophages (24).

In agreement with biological networks, the 4 expression studies that considered *GPX* (*GPX1*, *GPX2*, and *GPX3*), *GSR*, and *G6PD* all showed upregulation of each of these genes with smoking.

GSH Conjugation and Export

There were 24 association and 4 expression studies of Glutathione S-Transferases (GSTs), which play a role in GSH conjugation and export.

A homozygous deletion of *GSTM1*, resulting in a complete lack of activity (25), was associated with increased COPD risk in 3 of 7 association studies (range in OR: 2.2 to 8.0) (26-28). The prevalence of the deletion was increased in emphysema patients compared to non-diseased participants (29, 30), though no association was observed with emphysematous changes in heavy smokers (31). Both studies of chronic bronchitis reported a 3-fold increased risk associated with the null genotype (32, 33).

Five studies investigated the association of the *GSTM1* deletion with COPD-related quantitative traits. Conflicting evidence was reported: 1 of 2 studies of rate of

FEV_1 decline reported an association in men only (34) and 1 of 3 studies of FEV_1% predicted reported lower lung function with the null genotype (35). In a single study of FVC % predicted, the null genotype was significantly associated with decreased lung function (35). However, it was not associated with increased rate of FVC decline (34). Null genotype was associated with a steeper rate of FEF_{25-75} decline (among men) in one study (34).

In *GSTP1*, the Ile105Val substitution, which causes altered affinity for specific substrates (36), was associated with COPD-related outcomes. A protective effect of the heterozygous genotype was reported in 7 of 11 studies of COPD patients vs. asymptomatic participants; the magnitude of the effect varied and was statistically significant in 2 studies (37, 38). A study of smokers with emphysematous changes (vs. normal smokers) reported a protective effect of heterozygosity (31). However, 7 of 10 studies of the homozygous variant genotype in relation to COPD risk reported an increased risk: the difference was statistically significant in one study (39) and 4 estimates were based on small numbers.

The Ile105Val genotype had little or no relation to the rate of decline in FEV_1, although the direction of effect was consistent with the hypothesis: risk was increased in those homozygous for the variant allele (34). Greater effect sizes were found for risk of being in the tails of the FEV_1% predicted distribution (40), but there was little or no continuous relation with FEV_1% predicted (41).

A *GSTP1* polymorphism with unknown biological effect (Ala114Val) was investigated in 3 studies. One study of Indian smokers observed a statistically significant graded increase in prevalence of COPD with the variant allele (39), but a similar association with emphysema risk was not observed in an American population (30). In 3 comparisons of lung function within disease groups, statistically significantly lower lung function was observed with the variant allele in COPD

patients (39), but not in emphysema patients (41) or asymptomatic smokers (39).

There was little or no association of a homozygous deletion of *GSTT1* with COPD risk; 3 of 4 studies reported a slightly decreased risk of disease with the null genotype, but the interval estimates of the effect were wide. There was no association of *GSTT1* null and risk of emphysematous changes in smokers (31). Three of 4 studies of lung function reported an association: null genotype was associated with a steeper decline in FEV_1 in a general population (42), with steeper decline in FEV_1, FVC, and FEF_{25-75} among men (34), and with an increased risk of being in the lowest compared to the highest group of % predicted FEV_1 (40). The only study of mGST1 found no association between 4 markers and FEV_1 % predicted (41).

There were 3 studies of gene expression differences by disease group. Upregulation of *GSTM3* and *mGST1* expression was observed in COPD patients (18). *GSTO1* was significantly downregulated in the single study of lung tissue and sputum from COPD patients with lung tumor (43), but was upregulated in the epithelial cells of patients with COPD only (less severe stages) (18). Four studies investigated the expression of *GSTs* by smoking status. Both studies of *GSTA1* expression showed upregulation among smokers in lung tissue (18, 21). Statistically significant upregulation was associated with smoking in a single study of *GSTA2* (19). *GSTM3* expression was increased among smokers to the same extent (approximately 50%) in both studies of epithelial cells (18, 19). There was some evidence of upregulation of *mGST1* among smokers in 2 studies (18, 19). In 6 of 7 comparisons of *GSTO1* expression in various lung tissues, expression was unrelated to smoking.

Thioredoxin Metabolism

Thioredoxin metabolism was evaluated by considering the enzymes thioredoxin (TXN), thioredoxin reductase (TXNRD), and peroxiredoxin (PRDX). No

association studies and 2 expression studies were found. A single study considering epithelial cell expression in COPD vs. non-diseased reported upregulation of *TXN*, *TXNRD1*, and *PRDX1* with disease, and downregulation of *PRDX5* with disease (18). Both studies of expression by smoking status reported increased expression of *TXN* and *TXNRD1* with smoking (18, 19). In the study that also evaluated peroxiredoxins, *PRDX1* was upregulated and *PRDX3* and *PRDX5* were both downregulated in smokers (18).

Other Enzymes

Two classic antioxidants, superoxide dismutase (SOD) and catalase (CAT) were considered. Three association studies (evaluating 5 variants) and 6 expression studies were identified.

There was no association between an intronic SNP in *SOD1* and COPD. *SOD2* Val16Ala was associated with disease in a Chinese population (44), but not in persons of European descent (41). The association between *SOD3* Arg213Gly and COPD was studied in 2 large populations (45, 46). Heterozygosity was associated with a strong, statistically significant decreased risk of disease (~40-75% reduction)(45, 46) and a 70% reduction in risk of COPD hospitalization or death during follow-up (46). Genotype was not associated with lung function in a general population, but FEV_1/FVC ratio was higher among smokers with the heterozygous genotype ($p=0.04$) (46). There were no homozygous variants among diseased individuals in either study, precluding odds ratio calculation.

Three studies of SOD expression compared COPD patients to healthy controls. SOD activity was increased in the bronchial lavage fluid of nonsmokers with COPD compared to smokers with COPD and healthy controls (47). No evidence for differential expression by disease status was seen in 3 studies of SOD1 (18, 21, 48).

In the 6 comparisons of *SOD2* in lung tumor patients with COPD versus controls, COPD was associated with increased *SOD2* concentration (21, 48). An increase in expression was not observed in the single study of COPD patients without lung tumor (18). Neither of the studies of *SOD3* expression in disease groups provided strong evidence for differential regulation by disease (18, 21).

Little or no evidence of differential expression of *SOD1* and *SOD3* by smoking status was observed. *SOD2* was upregulated in smokers vs. nonsmokers in 3 comparisons of epithelial cells (with one showing strong, significant upregulation) and downregulated in 3 other comparisons. *SOD2* was also upregulated in lung tissue homogenate and alveolar macrophages, but downregulated in the pulmonary blood vessels of smokers vs. nonsmokers.

Two studies evaluated polymorphisms in *CAT*, but provided no strong evidence for an association of two promoter region SNPs with disease risk (44, 45). Two studies compared *CAT* expression in disease groups, with little evidence for differential regulation by COPD status, though statistically significant downregulation was observed in lung tumor patients with COPD in 1 study (21). Three studies compared *CAT* expression by smoking status, with inconsistent results.

Gene-Gene Interaction

Increased risk of COPD, and decreased FEV_1 % predicted among COPD patients, was reported for various genotype combinations that included either *GSTP1* 105Val or 114Val vs. wildtype for both polymorphisms (OR 1.99 for COPD risk; 95% CI 1.28, 3.09) (39). In an analysis of *GSTM1* null, *GSTT1* null, and *GSTP1* Ile105Val, most combinations of the "higher risk" genotypes were associated with an increased risk of disease, with the strongest associations observed with the *GSTP1* 105Ile allele and the null genotype for either *GSTM1* (OR 11.3; 95% CI 1.3, 98.6) or *GSTT1* (OR

12.1; 95% CI 1.3, 116.96) (37). Although the combination of *GSTM1* null, *GSTT1* null, and *GSTP1* 105Ile was not associated with COPD risk in another study (49), it was associated with steeper lung function decline (p=0.026)(40) and risk of rapid decline in lung function (OR 2.83; 95% CI 1.1, 7.2) (42). Men with the null genotype for both *GSTM1* and *GSTT1* had 8.3 ml/year greater decline in FEV_1 vs. those with at least one copy of both (p<0.001), with similar results reported for both FVC and FEF_{25-75} (34). The National Emphysema Treatment Trial reported little or no association between combinations of *GSTM1* null and *GSTP1* 105Ile and disease (30). There was little or no association of *GSTM1* and *GSTT1* null genotypes and emphysematous changes in Japanese heavy smokers (31). The combination of *GSTM1* null, *GSTP1* 105 Ile/Ile, and at least one slow allele for *microsomal epoxide hydrolase* increased risk of COPD (OR 6.8; 95% CI 1.6, 17.2) (26). The combination of *GSTM1* null and a *matrix metalloproteinase 9* polymorphism increased COPD risk by about 8-fold (OR 7.7; 95% CI 1.1, 53.3) (27).

Discussion

Observational epidemiologic evidence suggests a role for nutrients contributing to antioxidant function in the prevention of lung disease (4-9). Whether these findings reflect underlying biological mechanisms or methodologic bias (e.g., confounding) is unclear. The consideration of genetic variants that affect antioxidant/oxidant balance and that may be sensitive to dietary intake of antioxidants can help address this question. The study of genetic variants affecting antioxidant capacity allows an unbiased approach, in comparison to observational studies of diet, based in part on the principles of Mendelian randomization (50). Thus, this review was designed to evaluate the evidence that antioxidant enzyme function and/or regulation is related to COPD risk.

This systematic review included studies that addressed gene-disease associations as well as those that evaluated differences in gene expression. There were limited opportunities to synthesise results from both approaches as genes were often considered *either* in association studies *or* in expression studies, yet such synthesis may reveal complementary data (51). For example, a variant allele that leads to decreased glutathione was associated with an increased risk of COPD among smokers (12), and an expression study of *GCLM* reported upregulation in smokers (19). The combined results support the hypothesis that increasing available glutathione in persons with a high oxidant load may protect against lung damage. A lack of agreement between association and gene expression studies may also be informative. While association studies suggest a protective effect of heterozygosity for the *GSTP1* Ile105Val polymorphism in COPD, no differences in expression of *GSTP1* were reported in smokers compared to nonsmokers, suggesting that the effect of the genotype is not mediated through mRNA quantity.

Comparisons with animal studies provide an additional context in which to interpret the findings from human studies, but caution is warranted. Mice and adult rats (in contrast to humans) synthesise ascorbate (52), which protects GSH from oxidation and reduces it from its disulphide form (53). The interaction between ascorbate and other antioxidants suggests that animal studies of genetic manipulation or oxidant insult may not be predictive of results in humans. While two human studies of *GSR* expression reported significantly increased mRNA expression in the airway epithelial cells of asymptomatic smokers (18, 19), findings in smoke-exposed rats were mainly negative (54-56). Reduction of GSSG by endogenously formed ascorbate may blunt the rat's need for GSR to perform the same function.

This review reveals very few instances where the evidence base contains enough information to make a strong statement of effect, but a few examples deserve

mention. The *GSTM1* null genotype (no enzyme activity) was consistently associated with increased COPD risk (26-29, 32-35, 37, 57). A substitution in another GST, *GSTP1* Ile105Val, which affects catalytic activity and binding affinity for particular substrates, was consistently inversely associated with disease (31, 34, 37, 38, 40, 58-60). A rare substitution in *SOD3*, which increases plasma SOD levels, was also associated with a significantly decreased COPD risk (45, 46), a result supported by an animal study: transgenic mice overexpressing human *SOD3* had attenuated lung damage and inflammatory response in hyperoxic conditions (61). In addition, there was simultaneous upregulation of *GSR*, *GPX*, and *G6PD* in the airway epithelial cells of smokers (19), highlighting the importance of a network of genes in the lung's response to oxidative stress.

Several elements of the selected interrelated pathways have received minimal or no attention in human studies to date. For example, targeted disruption of the TXN gene produced early embryo lethality in mice (62, 63), and transgenic mice over-expressing human *TXN1* had increased survival and decreased hydroxyl radical production during exposure to diesel exhaust particles (64). The two studies of TXN-related enzymes in humans reported upregulation of *TXNRD1* and *TXN* in the airway epithelial cells of smokers and those with COPD (18, 19): further investigation is warranted. Other understudied genes of interest include *GGT*, *PRDX6*, and *GLRX*. GGT is the key enzyme in one pathway for the intracellular supply of cysteine for GSH synthesis: *GGT*-deficient mice show a reduced ability for *de novo* synthesis (65) and decreased intracellular GSH concentration (66). Furthermore, pulmonary *GGT* activity was increased during hyperoxia in rats (67), and *GGT*-deficient mice had worse survival in hyperoxic conditions (65, 66). PRDX6 is a peroxiredoxin that uses GSH as a cofactor. *PRDX6* null mice had more severe lung injury and significantly decreased survival in conditions of hyperoxia vs. wildtype (68). Transgenic mice

over-expressing *PRDX6* had greater survival and attenuated lung damage in hyperoxia (69). Finally, GLRX comprises part of a major thiol-disulphide redox buffer in the cell. The activity of this enzyme suggests a possible role in relation to the oxidation of GSH and the redox status of the cell, recommending it for further study.

Several methodological considerations deserve mention. COPD aetiology is expected to include gene-environment interactions, given the clear role of smoking in this disease and the inter-individual differences in response to cigarette smoke. Thus, comparison groups must be carefully selected with regard to smoke exposure. In association studies in which the non-diseased group is comprised of non-smokers and the diseased group of smokers, for instance, the estimate of a true effect may be diluted. Similarly, in expression studies, a comparison between individuals with equivalent exposures, but whose disease outcome differed may be most informative. Studies published in other languages were included to avoid the "Tower of Babel error" (70). A comprehensive set of enzymes based on biological networks were the starting point for the review, however our selections may have led to inadvertent omission of relevant enzymes. Disturbances in a broad range of redox-related enzymes are likely to affect disease risk, suggesting that complex interactions cannot be ignored. A broader network approach may ultimately lead to more robust epidemiologic findings.

In conclusion, the evidence summarised in this review supports the continued investigation of the hypothesis that variation in genes that code for enzymes that can alter the redox environment of the lungs may contribute to COPD risk. Future directions suggested by this summary are: more network-driven approaches that include broader consideration of enzymes whose related, redundant and linked activities might alter disease risk, further integration of association and expression studies to determine the nature of the biological relationships that may lead to disease,

and careful consideration, in both study design and analysis, of environmental exposures (e.g., smoking and nutritional status) that are likely to modify the gene-disease associations.

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CHAPTER 4

GENETIC VARIATION IN ANTIOXIDANT ENZYMES AND LUNG FUNCTION IN THE HEALTH, AGING, AND BODY COMPOSITION STUDY

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Abstract

Not all cigarette smokers develop chronic obstructive pulmonary disease (COPD), and discovering susceptibility factors is a high research priority. The oxidative burden of smoking may overwhelm antioxidant defenses if those defenses are compromised as a result of sequence variants in genes encoding antioxidant enzymes. This study explores the association between genetic variation in a broad network of antioxidant enzymes and lung phenotypes. Linear models were used to evaluate associations among 2,387 European and African American seniors in the Health, Aging, and Body Composition (Health ABC) Study. Genes encoding members of the peroxiredoxin and isocitrate dehydrogenase gene families were in the most statistically significant SNPs list and relatively common variant genotypes were associated with biologically meaningful differences in phenotype, suggesting these genes, especially *PRDX5*, *PRDX3*, *IDH3A*, and *IDH3B*, as targets of future research. After correction for multiple comparisons, 4 associations remained statistically significantly associated with the ratio of FEV_1/FVC in African Americans, as follows: interactions between a SNP in *IDH3B* with both smoking status and smoking dose (p_{adj}=0.002 and 0.01, respectively), and interactions between 2 SNPs in a glutaredoxin, *GLRX2*, with smoking dose (p_{adj}=0.03 for both). These genotypes have not been investigated in previous candidate gene association studies, and thus the findings suggest novel mechanisms and targets for future research and provide evidence for a contribution of sequence variation in antioxidant enzymes to COPD risk.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by the development of airflow obstruction that is not fully reversible, a phenotype that is characteristic of chronic bronchitis and emphysema. At advanced stages, COPD commonly progresses to respiratory failure and death, and is a significant cause of morbidity as well as the fourth leading cause of mortality in the U.S.(1). Airflow obstruction is reliably and validly measured by spirometry, specifically by the ratio of the forced expiratory volume in the first second to the forced vital capacity (FEV_1/FVC) and by the reduction in percent of the FEV_1 value predicted according to an individual's age, height, race, and gender ($ppFEV_1$). Both spirometry parameters are used to diagnose and stage COPD (2). Decline in FEV_1 occurs naturally with aging, but a steeper rate of decline, as observed in susceptible cigarette smokers, is a harbinger of the debilitating low lung function that characterizes COPD.

Current theories of the pathogenesis of COPD posit that an imbalance between oxidant burden and antioxidant protection leads to oxidative damage that contributes to disease pathogenesis. Diminished antioxidant defenses, especially among cigarette smokers, who are exposed to a high level of oxidants, are hypothesized to contribute to tissue changes underlying disease development (3). A minority of smokers develop obstructive lung disease(4), consistent with the hypothesis that in some smokers compromised antioxidant protection, as a result of genetic variation in antioxidant enzyme and/or diets low in antioxidants, contributes to susceptibility. While observational studies mainly report positive associations between nutrients with antioxidant properties and lung function, with stronger effects in cigarette smokers, studies of genes that play a role in antioxidant defenses provide a novel test of the susceptibility hypothesis.

Genetic studies show that lung function is a heritable trait, and heritability

estimates of FEV_1 and the ratio of FEV_1/FVC range from 40-90% (5-7). A single gene disorder that increases COPD risk, alpha 1-antitrypsin deficiency, has been discovered, but it accounts for only about 1 to 2% of cases(8), suggesting the importance of additional genes in lung function variability. Recently completed genome-wide association studies of lung function phenotypes (9-11) have identified 14 genomic regions for further investigation. Well-designed studies of candidate genes, specifically of genes encoding antioxidant enzymes, are important to the evidence base, but published studies have several limitations (as reviewed in (12)). The majority of candidate gene studies consider only a few markers and/or genes, and thus fail to capture the related and redundant functions within the network of antioxidant-related genes. Also, by definition, such studies focus on a limited subset of genes (most prominently, glutathione S-transferases), thus many genes related to antioxidant defense are unstudied at the population level (for example, genes of the thioredoxin system: thioredoxin, thioredoxin reductase, and peroxiredoxin). Studies of the heritability of lung function provide compelling evidence for a gene-environment interaction (13-15), specifically a gene by smoking interaction, and a recent genome-wide assessment of gene expression in the small airway epithelium and genotype in smokers and non-smokers indicates considerable influence of smoking parameters modifying the relation between genotype and gene expression (Andrew Clark, personal communication). Despite this evidence, many studies do not consider gene-environment interactions and/or do not appropriately account for cigarette smoking in study design and analysis. Finally, data on African Americans are limited, and most studies with African American participants are underpowered to investigate any differences in the effects of genotype on lung phenotype by race.

We investigated the association of variants in genes encoding antioxidant enzymes with the lung function phenotypes ppFEV_1 and FEV_1/FVC. As the elderly

are at the greatest risk for reduced lung function associated both with aging and smoking, these analyses were conducted in the Health, Aging, and Body Composition (Health ABC) Study, a prospective cohort of African American and European American seniors. The discovery-based approach explored the relation of all genotypes with the two phenotypes, stratified by race, and allowing for differential effects of genotype by cigarette smoke exposure and dose.

Methods

Population

The Health ABC study cohort comprises 3,075 older men and women (aged 70-79 at baseline). Health ABC is a random sample of European Americans and all African American Medicare-eligible persons residing in ZIP codes from the metropolitan areas surrounding Pittsburgh, Pennsylvania and Memphis, Tennessee. Eligibility criteria included self-report of: no difficulty walking one-quarter of a mile or climbing 10 steps without resting; no difficulty performing basic activities of daily living; no use of a cane, walker, crutches or other special equipment to ambulate; no history of active treatment for cancer in the prior 3 years; and no plan to move out of the area in the subsequent 3 years.

Exclusion criteria included a low call rate for genotypes, missing outcome measurements, or prevalent chronic obstructive pulmonary disease, defined as *both* FEV_1 and FEV_1/FVC below the population-defined lower limits of normal. Exclusions were also made based on quality of spirometry testing for FEV_1; participants with low quality FVC measurements were additionally excluded from the FEV_1/FVC analysis.

Lung Function Outcome

Spirometry was performed during the clinical visit at study entry using a

horizontal dry rolling seal HF6 Spirometer (Sensor Medics Corporation, Yorba Linda, CA) connected to a personal computer. Tests were conducted in accordance with standardized guidelines, as previously reported (16). $PpFEV_1$ values were calculated using prediction equations generated from the Third National Health and Nutrition Examination Survey data (17). When pulmonary function measurements from the first clinical visit were not available or not acceptable, the first acceptable spirometry data from a subsequent clinical visit (at 4 and 7 years of follow-up) was included, provided that the age of the participant at the time of the measurement was within the age range of the full sample at the first clinical visit (age 70-79). The majority (95%) of data used in this analysis was from the first clinic visit.

Selection of SNPs

Fifty-six genes were identified that are known to affect the balance of antioxidants/oxidants and are expressed in lung tissue (Table A.4). 384 SNPs were selected with the goal of capturing variation across each gene and its regulatory region (2 kb upstream and downstream); further details of analyzed SNPs are presented separately (Table A.7). SNP selection was conducted according to the following order of priority: 1. nonsynonymous SNPs, 2. SNPs to provide adequate coverage of genetic variation (maximum LDU of 0.9 LDU between SNPs), 3. if genetic variation was limited across the gene, SNPs to cover large physical distance across the gene, and 4. SNPs in high LD with SNPs of interest to provide redundancy in the event of assay failure. When possible, a minimum of 5 SNPs were selected per gene. Separate consideration was given to European American and African Americans in SNP selection to maximize coverage in both populations, given differences in LD structure and allele frequencies.

Genotyping

DNA for the Health ABC participants was extracted using the Gentra Puregene

DNA Purification Kit (QIAGEN, Valencia, CA) from stored, frozen buffy coat originally extracted from 10 ml whole blood. SNPs were assayed using the Illumina Goldengate platform; assays were performed by Johns Hopkins University SNP Center, the Center for Inherited Disease Research, under the auspices of the National Heart, Lung, and Blood Institute's Resequencing & Genotyping Service. Genotyping quality was excellent as determined through use of blind duplicates and HapMap controls with known genotypes (99.99% and 99.83% reproducibility rates, respectively).

Data Analysis

Linear models were used to evaluate the association between SNPs and lung function phenotype. All models included adjustment for study site, age, height, and gender (18). SNPs were coded as additive, recessive, dominant, or heterozygote advantage, depending on the pattern of association observed and a sufficient number of participants in subgroups of genotype and smoking parameters. SNPs with a minor allele frequency <1% were excluded from the analyses (n=29 in European American; n=1 in African Americans).

Smoking status was represented as former and current smokers, separately, compared to never smokers (defined as self-report of less than 100 cigarettes smoked throughout lifetime). Smoking dose was considered as packyears, and categorized as high (above the median, 27 packyears), low (1-27 packyears), or no smoking history. Modification of the SNP-outcome association by smoking was assessed through product terms of each SNP with both smoking dose and smoking status.

As a first approach, regression coefficients with a nominal $p<0.002$ were selected as the most statistically significant 'hits' or findings. In further analyses, correction for multiple comparisons was conducted as follows: within an analysis, p-values were adjusted using the false discovery rate with a q-value of 0.05 (19); p-

values were then multiplied by the number of analyses of that phenotype in order to adjust for correlated outcomes. For example, the false discovery rate adjustment was applied to all of the results for European Americans testing the interaction between SNPs and smoking status in the prediction of $ppFEV_1$; these p-values were then multiplied by 3 to account for the number of analyses conducted on this outcome (main effects of SNP on $ppFEV_1$, interaction between SNPs and smoking status on $ppFEV_1$, and interaction between SNPs and smoking dose on $ppFEV_1$). Both approaches, nominal $p<0.002$ and multiple comparison adjusted findings are presented in results. R^2 values between SNPs were determined using Haploview (20). All data analyses were conducted using SAS v 9.1 (SAS, Cary, NC).

Results

Genotype data were available for 2,762 participants. After exclusion for missing smoking information (n=5), 2,387 (86%) participants had an acceptable FEV_1 measurement and were included in the $ppFEV_1$ analysis, and 2,190 (79%) had acceptable FVC measurements and were included in the FEV_1/FVC analysis (Table 4.1). The $ppFEV_1$ phenotype had a mean of 97% in this population, with a range of 36% to 181% and a standard deviation of 19%. The ratio of FEV_1/FVC phenotype (calculated as FEV_1/FVC * 100) had a mean of 75, with a range of 34 to 98 and a standard deviation of 6.

The 35 most statistically significant SNPs (nominal $p<0.002$) reveal more effects in African Americans, and only 10 (29%) of the top hits were in European Americans. Nine of the most statistically significant SNPs (26%) were associated with the $ppFEV_1$ phenotype. Only 7 of the associations (20%) were in models estimating the main effect of the SNP on lung function phenotype (Table 4.2), and the majority of statistically significant associations were in models of effect modification

Table 4.1: Characteristics of Health, Aging, and Body Composition Study Participants Included in Analyses[a]

	% Predicted FEV_1		FEV_1/FVC	
	African Americans n=972	**European Americans n=1415**	**African Americans n=855**	**European Americans n=1335**
Age, years	73.3 (2.9)	73.7 (2.8)	73.3 (2.9)	73.7 (2.9)
Women (%)	569 (58.5)	682 (48.2)	481 (56.3)	629 (47.1)
Memphis, TN site (%)[b]	434 (44.7)	674 (47.6)	375 (43.9)	638 (47.8)
Former Smokers (%)	390 (40.2)	707 (50.0)	350 (41.0)	668 (50.1)
Current Smokers (%)	139 (14.3)	75 (5.3)	124 (14.5)	71 (5.3)
Packyears	27.4 (23.9)	34.3 (30.9)	27.7 (23.9)	34.2 (30.8)
FEV_1, ml				
Women	1724 (367)	1946 (379)	1729 (368)	1959 (376)
Men	2369 (517)	2759 (531)	2382 (511)	2767 (533)
$PpFEV_1$	99.4 (21.4)	95.5 (16.9)	99.8 (21.3)	95.9 (16.9)
FEV_1/FVC	77.0 (7.4)	75.9 (6.1)	76.0 (6.6)	75.1 (5.8)

[a] Mean (SD) listed unless otherwise indicated.
[b] vs. Pittsburgh, PA site.

Table 4.2: Main Effects, Most Statistically Significant Results[a]

Population	Outcome	SNP[b]	Gene	P-value[c]	Key Findings
European	$ppFEV_1$	None			
Americans	FEV_1/FVC	None			
African	$ppFEV_1$	Rs535537	*GSTM4*	0.0007	AA 14% higher, AG 4% lower than GG[d]
Americans		Rs627365	*GSTM4*	0.001	AA 43% higher, AG 5.5% higher than GG
		Rs7768	*PRDX3*	0.001	CC 5% lower than CG/GG
	FEV_1/FVC	Rs2284650	*GCLC*	0.002	GG ratio 9 lower than GA/AA
		Rs7294985	*mGST1*	0.0004	AA/AG ratio 2.3 lower than GG
		Rs7768	*PRDX3*	0.001	CC 2% lower than CG/GG
		Rs7068937	*PRDX3*	0.002	AA/AG 1.4 higher ratio than GG

[a]All results with nominal p-value <0.002

[b]SNPs in **bold** remained statistically significant after adjustment for multiple comparisons

[c]Nominal p-values shown

[d]Participants with the homozygous variant genotype had 14% higher ppFEV1 than participants who were homozygous for the wild type allele. Heterozygotes had a 4% lower $ppFEV_1$ than wild type homozygotes.

of the SNP by cigarette smoking (Table 4.3). Effect sizes ranged from 4% to 35% for the $ppFEV_1$ phenotype, and from 1.4 to 15 units for the FEV_1/FVC ratio phenotype. Together, the most statistically significant SNPs associated with the main effects accounted for 3% of the variability in the $ppFEV_1$ phenotype and 4% of the variability in the FEV_1/FVC ratio phenotype among African Americans (no main effect SNPs were identified in European Americans). SNP-smoking interactions accounted for 3% (both European and African Americans) of the variability in the $ppFEV_1$ phenotype, and 5% and 17% (European and African Americans, respectively) of the variability in the FEV_1/FVC ratio phenotype.

SNPs in genes encoding peroxiredoxins (*PRDXs*) were associated with $ppFEV_1$ and FEV_1/FVC in both African Americans and European Americans. An interaction between rs9787810 (*PRDX5*) and both smoking status and smoking dose was highly statistically significant in models of both lung phenotypes in European Americans. Although slightly less statistically significant (and not shown), the interactions between another SNP in *PRDX5* (rs1047206) and both smoking status and dose were associated with ppFEV1 in African Americans (p=0.004 for both). In African Americans, SNPs in *PRDX3* (rs7768 and rs7068937) were associated with lung phenotypes, and a third SNP (rs1119881; R^2 for all 3 SNPs <0.8) had a similar though less statistically significant association (p=0.004). A SNP in *PRDX4* (rs528960) modified the effect of smoking status on the ratio of FEV1/FVC in African Americans. Overall, 6 of the 19 SNPs in *PRDX3*, *PRDX4*, and *PRDX5* were associated with lung outcomes at $p<0.005$.

The association of SNPs in isocitrate dehydrogenase (*IDH*) genes with lung function phenotypes comprised some of the most statistically significant findings. Three *IDH* SNPs in European Americans (2 in LD) and 5 *IDH* SNPs in African Americans (none in LD) showed evidence of interaction with cigarette smoke in

Table 4.3: Smoking Interactions, Most Statistically Significant Results[a]

Population	Outcome	Interaction[b]	Gene	P-value[c]	Key Findings[d]
European Americans	$ppFEV_1$	Rs17674205*Status	*IDH3A*	0.0008	FS GG 39% higher than FS GA/AA (no CS GG)[g]
		rs9787810* Status	*PRDX5*	0.0002	FS AA/AG 12.7% higher than FS GG
		Rs6413428*Status	*SEPP1*	0.001	CS GG 43% higher than CS GA/AA; FS GG 14% higher than FS GA/AA
		Rs9787810*Dose	*PRDX5*	0.002	FS AA/AG high dose 11%, low dose 8.5% higher than FS GG
	FEV_1/FVC	Rs11631100*Status[e]	*IDH3A*	0.0009	CS AA 12.7% higher than CS AG/GG
		Rs3816253* Status[e]	*IDH3A*	0.001	CS GG 12.5% higher than CS GA/AA
		Rs9787810* Status	*PRDX5*	0.0002	FS AA/AG ratio 4.5 higher than FS GG
		Rs2066511*Dose	*GCLC*	0.0006	CS AG ratio 3 higher (low dose) than CS AA/GG
		Rs3740466*Dose	*GSTO2*	0.002	CS AG ratio 4.6 and 2.3 lower (high/low dose) than CS AA/GG
		Rs9787810*Dose	*PRDX5*	0.002	FS AA/AG ratio 4 higher (high and low dose) than FS GG
African Americans	$ppFEV_1$	Rs1002149*Status	*GSR*	0.0006	FS AC 19.5% higher than FS AA/CC
		Rs4236107*Status	*GSTA5*	0.002	FS GA 9% higher than FS GG/AA
	FEV_1/FVC	Rs8030346*Status	*IDH2*	0.0009	CS GG ratio 1.2 lower than CS GA/AA
		Rs6107100*Status	*IDH3B*	0.000002	CS AC ratio 13 higher than CS AA/CC
		Rs6037255*Status	*IDH3B*	0.002	CS AA/AC ratio 13 higher than CS CC
		Rs528960*Status	*PRDX4*	0.002	CS GA ratio 9 higher than CS GG/AA
		Rs2042286*Status	*SEPW1*	0.0002	CS AA/AG ratio 15 higher than CS GG
		Rs1139793*Status	*TXNRD2*	0.0007	CS AA/AG ratio 2.5 lower than CS GG
		Rs10801174*Dose[f]	*GLRX2*	0.0001	CS TT/TA ratio 10 higher (high and low dose) than CS AA
		Rs7547615*Dose[f]	*GLRX2*	0.00009	CS GG/GA ratio 10 higher (high and low dose) than CS AA
		Rs2250192*Dose	*GSR*	0.0005	CS AA ratio 11 lower than CS CC
		Rs2281594*Dose	*GSTA3*	0.002	CS CC ratio 11.5 lower (low dose) than CS CG/GG
		Rs8028234*Dose	*IDH2*	0.001	CS TT/TA ratio 7/13 (high/low dose) higher than CS AA
		Rs17674205*Dose	*IDH3A*	0.001	CS GG/GA ratio 13 higher (high dose) than CS AA
		Rs6107100*Dose	*IDH3B*	0.00001	CS AC ratio 11/10 (high/low dose) higher than CS AA/CC
		Rs2297765*Dose	*mGST3*	0.001	CS GG ratio 14 lower than CS GA/AA
		Rs2042286*Dose	*SEPW1*	0.001	CS AA/AG ratio 12 higher (high and low dose) than CS GG
		Rs8139906*Dose	*TXN2*	0.001	CS CC/CG ratio 12 higher (high) than GG; FS CC/CG ratio 2.7 lower than FS GG

[a]All results $p<0.002$, [b]SNPs in **bold** statistically significant after multiple comparison adjustments,[c]Nominal p-values, [d]Abbreviations: CS (current smokers); FS (former smokers), [e]LD observed between SNPs ($R^2=0.92$) [f]LD observed between SNPs ($R^2=0.99$) [g] FS homozygous for the variant genotype (GG) had a 39% higher $ppFEV_1$ than FS who were heterozygous (GA) or homozygous for the major allele (AA). No CS were homozygous for the variant genotype.

predicting lung phenotypes. The most statistically significant finding in these analyses was the interaction of rs6107100 (*IDH3B*) and smoking status in relation to the FEV_1/FVC ratio ($p=2 \times 10^{-6}$). Ten additional associations between SNPs in *IDH* genes and lung phenotypes were statistically significant at $p<0.005$ (data not shown). SNPs in genes with disulfide reductase activity were also prominent among the most statistically significant findings in African Americans, and interactions between smoking and SNPs in *GSR*, *GLRX2*, and *TXNRD2* were identified.

After correction for multiple comparisons, 4 results remained statistically significant (indicated in bold in Table 4.3). The *IDH3B* SNP (rs6107100) modified the effect of smoking status and dose on the ratio of FEV_1/FVC in African Americans ($p_{adj}=0.002$ and 0.01 for SNP by smoking status and dose interaction terms, respectively). Two SNPs in *GLRX2* (rs10801174 and rs7547615, in strong LD) also modified the effect of smoking dose on the ratio of FEV_1/FVC in African Americans ($p_{adj}=0.03$ for both).

Some of the most statistically significant results were excluded from presentation here due to small numbers of participants ($n<5$) in the genotype/smoking subgroup with the strongest association. Despite the reduced confidence in these results, the strength of the associations observed (some of which remained statistically significant after correction for multiple comparisons) may suggest a biologically interesting relation. These results are summarized separately (Table A.8). Briefly, SNPs in genes associated with glutathione synthesis (*GCLC* and *GGT*), with peroxidase activity (*GPX2*, *GPX3*, *PRDX6*), and encoding glutathione S-transferases (*GSTA3*, *GSTA5*, *GSTZ1*, and *mGST2*) were associated with the lung function phenotypes studied.

Discussion

This study was designed to evaluate the hypothesis that sequence variation in genes encoding antioxidant enzymes, which are expected to alter antioxidant defenses, contributes to COPD susceptibility, especially among cigarette smokers. The findings reported here support this hypothesis and provide important direction for future research.

SNPs in the peroxiredoxin family of genes were prominent in the list of most statistically significant associations. Interactions between SNPs in *PRDX5* and smoking parameters were associated with $ppFEV_1$ in both African Americans and European Americans and with FEV_1/FVC in European Americans. For rs9787810, a *PRDX5* SNP appeared 4 times in the top results, and both the size of the effect (about 12% higher on $ppFEV_1$ and 4 units higher on ratio of FEV_1/FVC in the AA/AG genotype groups vs. wildtype) and the prevalence of the variant genotypes in the European ancestry group (54%) suggest that this finding is important in terms of population attributable risk. However, these particular genotypes are less prevalent in African Americans (13%), and small numbers in genotype/smoking subgroups limited analyses. In African Americans, SNPs in another peroxiredoxin gene, *PRDX3* (rs7768, rs11198811, rs7068937), were associated with moderate effect sizes (5% and 4% lower $ppFEV_1$, and 1.4 units higher ratio, respectively, with the variant genotypes as represented in Table 4.2), for genotypes that are relatively common (21%, 23%, and 33%), suggesting that these associations also are meaningful in terms of population attributable risks.

Peroxiredoxins, also called thioredoxin reductases, are a family of peroxidases that use the reducing power of thioredoxin (with the exception of PRDX6, which uses glutathione). Although, this is the first study of genetic variants in these genes and lung phenotypes, several prior microarray studies provide relevant context for these

results. Both *PRDX3* and *PRDX5* were downregulated in smokers compared to never smokers (21). In *PRDX3* knockout mice, excessive production of reactive oxygen species (ROS) from macrophages was observed after exposure to lipopolysaccharide, an inflammatory agent from gram-negative bacteria (22). Clearly further study of peroxiredoxins and COPD-related lung outcomes is needed.

Members of the isocitrate dehydrogenase gene family (*IDHs*) were also among the most statistically significant associations, and SNPs in these genes interacted with smoking to predict lung phenotypes in both African Americans and European Americans. The effect sizes for SNPs in these genes were large, and differences between the variant and reference genotypes range from 13 to 39% higher ppFEV_1 for associations with *IDH3A*, and differences of over 10 units in the ratio of FEV_1/FVC for *IDH3A*, *IDH3B*, and *IDH2* (with the exception of an inverse association for the interaction between smoking status and rs8030346 on FEV_1/FVC). The majority of these SNPs are common, variant genotype prevalence ranges from 15 to 63% (only 2 SNPs have a prevalence of < 10% [rs8030346 (*IDH2*) and rs17674205 (*IDH3A*)]), making the potential impact on lung function outcomes among smokers of considerable interest. While these genes are relatively unstudied at the population level as contributors to antioxidant defenses, the role they play in supplying the reducing equivalents for the antioxidant activity of the many members of the glutathione and thioredoxin system could be pivotal in determining cellular redox balance. In rats, IDH activity and protein expression was age-dependent (23), a finding of interest in relation to the associations reported here for phenotypes related to the aging of the lung. In fibroblasts, decreased expression of *IDHs* led to higher lipid peroxidation, oxidative DNA damage, intracellular peroxide generation, and increased senescence, indicating an important regulatory role for these genes in the defense against oxidative stress (24).

The SNPs identified in our top results, and their interactions with smoking parameters, accounted for a relatively large proportion of the variability observed in these phenotypes. The four demographic variables, age, height, gender, and study site together explained 5% and 14% (European and African Americans, respectively) of the $ppFEV_1$ phenotype, and 2% and 3% (European and African Americans, respectively) of the FEV_1/FVC phenotype. Smoking variables explained an additional 4-7 % of both phenotypes in both European and African Americans. In the context of these known predictors of lung function, the 3-4% of variability associated with SNPs in main effect models and 3-17% of variability associated with SNP-smoking interactions provide compelling support for a role of these variants in predicting lung function.

None of the actual SNPs in our top results have been investigated in published association studies of lung function, and only 2 previous association studies investigated other markers in genes represented in our top results. The variant genotype of a SNP in a glutathione synthesis gene, *GCLC*, was associated with an increase in COPD risk in a Chinese population [OR 1.8 (95% CI: 1.0, 3.3)](25). This SNP (rs17883901) was included in our analyses, but no association was observed. The variant allele has a higher prevalence among Asians (13%) than among African Americans (1%) or Europeans (6%), suggesting a potential reason for this discrepancy. SNPs in a glutathione S-transferase, *mGST1* (rs11875, rs2160512, rs2239675, and rs2287152), were not associated with $ppFEV_1$ in European American emphysema patients (26). None of these SNPs were included in our analyses, nor were they in LD with the SNP in this gene which we found associated with lung function (rs7294985)**.** SNPs included in this analysis were in LD with the previously investigated SNPs, and agreed with the null findings.

In the list of most statistically significant findings (nominal $p<0.002$), a

striking 50% of genes, comprising 66% of results, encode enzymes found in mitochondria. The mitochondrial genes include all of the *IDH* genes (*IDH3A*, *IDH3B*, and *IDH2*) and 2 of the 3 *PRDX* genes (*PRDX3*, and *PRDX5*) in the most statistically significant results. To date, there are no studies of the role of mitochondrial function in relation to lung function at the population level, but results from basic science studies provide a useful context for interpretation. A recent series of experiments addressed the effect of cigarette smoke on lung epithelial cells. While systemic increases in biomarkers of oxidative stress are observed with smoking, the high concentration of reactive oxygen species (ROS) in cigarette smoke are in the gaseous phase; these components likely assault the lining fluid and plasma membranes of epithelial cells, but cannot enter cells directly, which leaves the mechanism by which they contribute to systemic oxidative stress unclear. A recent paper suggests that *lipophilic* compounds in cigarette smoke cross through the plasma membrane and induce mitochondrial overproduction of ROS, which would be expected, in turn, to increase systemic (and local) oxidative stress(27). In studies of mouse embryonic fibroblasts, oxidizing extracellular conditions induce mitochondrial overproduction of ROS, signaling an antioxidant response (through nuclear factor (erythroid-derived 2)-like 2, Nrf2). Embryonic fibroblasts from transgenic mice over-expressing *TXNRD2* produced less intracellular ROS in oxidizing extracellular conditions, resulting in diminished Nrf2-regulated antioxidant gene expression (28). Also of interest, particulate matter, another inhalant associated with lung function decrements, induces mitochondrial oxidant overproduction, which leads to the alveolar epithelial cell apoptosis associated with this exposure. Overexpression of the gene encoding a cytoplasmic antioxidant enzyme (*SOD1*) prevented the excess ROS generation and subsequent cell death (29). Taken together, these results suggest a key role for mitochondria and mitochondrial antioxidant enzymes in the lung and systemic

response to cigarette smoke exposure. Specifically, the lipophilic portion of cigarette smoke and the oxidizing extracellular environment (the epithelial lining fluid after depletion of antioxidants resulting from direct contact with ROS in cigarette smoke) may induce an excess production of ROS in the mitochondria; any variability in mitochondrial antioxidant enzyme expression or function as a result of sequence variation, then, would have significant effects on the oxidative burden in the lungs (and beyond).

A larger proportion of statistically significant results were consistently observed in African Americans compared to European Americans. Potential explanations for this include the following: different distribution of the phenotype in the two populations, different exposure to cigarette smoking, and differences in genetic variation in the studied genes. Mean $ppFEV_1$ was higher in African Americans, and the standard deviation was larger compared to European Americans; the mean FEV_1/FVC ratio was also somewhat higher in African Americans, and the standard deviation was greater (Table 4.1). More variability in the phenotype could improve the ability to distinguish different effects by genotype, but it is unlikely that these small differences fully account for the dramatic differences observed by race. A much higher proportion of African Americans were current smokers (14% compared to 5% in European Americans), although their smoking history suggests a lower lifetime dose (27 vs. 34 packyears in African vs. European Americans, respectively). The higher proportion of participants who are currently smoke-exposed may indicate a greater burden of oxidative stress on these antioxidant enzymes, making the effects of perturbations to these enzymes (via genetic variants) more apparent. Finally, as is well-established, patterns of linkage disequilibrium are very different between African Americans and European Americans as a result of migratory history of populations. The reduced correlations between SNPs in African Americans functionally equates to

a greater level of variation tested. This increase in genetic variation tested could reasonably enhance the ability to detect associations. Only one SNP was identified among the most statistically significant results in both African Americans and European American: interactions between smoking parameters and rs1764205 (*IDH3A*) were associated with lung outcomes; these results were in a consistent direction, with large increases in lung function observed in cigarette smokers with variant genotypes. Despite the lack of overlap, however, the same functional gene groups were prominent in both African American and European American subgroups (peroxidase activity, supply of reducing equivalents, and disulfide reductases).

There were consistently more statistically significant associations with the ratio of FEV_1/FVC phenotype as compared to ppFEV_1. As exemplified by the role of the ratio in diagnosing COPD, reductions in the ratio are a clear indicator of airflow obstruction. Using an "adjustment" of the FEV_1 value for FVC (via calculation of the ratio) essentially isolates the factors that are associated with obstruction and not with overall lung capacity.

Looking separately for the interactions between smoking status and dose allowed for some inference regarding the potential role of an enzyme in a smoking challenge. For instance, interactions were observed for SNPs in *IDH* genes with both smoking status and smoking dose; this result suggests that these genes are broadly important for both smoke exposure and increasing levels of smoking. SNPs in *GSR*, however, were only observed to interact with smoking dose, suggesting a differential effect for varying levels of exposure.

Several strengths of this work deserve mention. First, these analyses were conducted in a large, epidemiological cohort, a study design which is uniquely suited to investigate the genetics of complex diseases, especially when considering gene-environment interactions (30,31). Other aspects of the design of the cohort were

advantageous to this work: the advanced age of the participants, which meant greater variability in the outcome as a result of both aging and environmental exposure; careful characterization of smoking history; a sufficiently large proportion of African Americans to justify a stratified analysis (a relatively undescribed population with respect to this research question); and high quality spirometry.

Currently, researchers are turning to genome-wide association studies (GWAs) to discover novel gene regions of interest in relation to a particular phenotype. The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium recently completed a meta-analysis of GWAS of FEV_1 and FEV_1/FVC in over 20,000 participants in 4 epidemiologic cohort studies, including Atherosclerosis Risk in Communities, Cardiovascular Health Study, Framingham Heart Study, and the Rotterdam Study was recently completed (32). None of the genes in our most statistically significant results list (nor any of our studied genes) appeared in the top 2000 hits in the CHARGE study. According to a recent study, it is unlikely that this discrepancy is a result of the failure of the GWAS to appropriately capture the genetic variation present in our studied genes (33). Although a replication of the Health ABC findings by the CHARGE cohorts would strengthen the finding, an absence of replication may be explained by several scenarios. The CHARGE GWAS considered a linear, additive coding of SNPs, such that 2 copies of an allele were expected to have an effect size that was approximately double the effect of a single copy of the allele, and a single parameter estimate was used to describe this relation (thus, regression coefficients and p values test this additive effect). In the Health ABC analyses, the best coding for each SNP (on the basis of strength of association with outcome) was used, and additive coding was modeled as a set of dummy variables to avoid assumptions about linearity in the genotype—phenotype association. Given that SNPs are known to affect disease risk in a variety of ways (i.e. dominant, recessive,

heterozygote advantage, additive/multiplicative), the lack of replication may be explained by a difference in coding between CHARGE and Health ABC studies. Although the CHARGE GWAS stratified by smoke exposure (with never smokers analyzed separately from current and former smokers), there was no formal test of gene-environment interactions (for instance, with a product term between the SNP and smoking parameter), another difference between CHARGE and Health ABC analysis approaches.

When the models producing the 35 most statistically significant results in the Health ABC analyses were re-analyzed using the coding and stratification strategies described in the CHARGE GWAS, only 2 of the 35 SNPs remained associated with the outcome at the level of statistical significance used to define the set of top hits ($p<0.002$). Only one of these results (Rs6037255 in the prediction of FEV_1/FVC among smokers) would have met the CHARGE GWAS criteria for inclusion among the top 2000 hits for this outcome; seven of the results reported among the most statistically significant results in the Health ABC analysis as presented met this criteria. Clearly, decisions regarding coding of alleles and modeling of gene-environment interactions are of vital importance in genetic association studies.

Another notable difference between the Health ABC study and the CHARGE GWAS meta-analysis is the demography of the populations studied. The Health ABC population was about 40% Black, while all of the participants in the CHARGE analyses were of European ancestry (an important difference considering the proportionately higher number of findings among Blacks in the Health ABC results). Also, the mean age of the participants in the CHARGE GWAS was considerably lower (58 vs 73 years); a smaller proportion of these populations may be experiencing the age-related increases in susceptibility to low lung function. Consistent with this hypothesis, the mean FEV_1 was higher in CHARGE (2,807 vs 2,215 ml), a difference

that could not be attributed to differences in gender distribution (mean proportion of women 55 and 52% in CHARGE and Health ABC, respectively). Population characteristics are also of vital importance in drawing comparisons between genetic epidemiology studies.

Although overlap exists in this project and the CHARGE GWAS analysis, these differences above highlight the unique contribution of the Health ABC project in addressing the question of genetic factors in COPD susceptibility: motivating candidate gene studies with biological hypotheses allowed for a more focused interrogation of the role of these genes and consideration of appropriate environmental factors expected to interact with them.

In summary, this analysis provides evidence for a role of genetic variation in antioxidant enzymes and lung function outcomes important in predicting risk of COPD and the trajectory of aging. This work complements research conducted on dietary determinants of antioxidant capacity in that the genetic determinants of antioxidant capacity are not anticipated to be associated with lifestyle factors, and so are not subject to the residual confounding concerns that limit the interpretation of studies of diet. Of particular importance in these results are SNPs encoding members of the peroxiredoxin and isocitrate dehydrogenase gene families, suggesting that the function of peroxidases dependent on thioredoxin and the overall production of reducing equivalents are important in predicting lung outcomes, especially in the presence of oxidant challenge, and interventions that support these systems may yield beneficial results.

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CHAPTER 5

CONCLUSION

The three projects comprising this dissertation focus on the role of antioxidant defenses in maintaining lung health and in avoiding chronic obstructive pulmonary disease (COPD), particularly with oxidative stress challenge. Specifically, the overarching goal of this research is to understand whether variability in antioxidant defenses contributes to the susceptibility to COPD and lower lung function, especially in the presence of cigarette smoke. There are at least three determinants of the balance between oxidants and antioxidants in the lung: *environmental* oxidant exposures (exogenous sources of oxidants such as smoking, occupation, air pollution), and endogenous capacity to offset damage by oxidants as influenced by *dietary* intake of nutrients with antioxidant properties, and by *genetic* variation affecting antioxidant-related enzymes. The projects described herein address the roles of environment, nutrition and genetics in relation to lung health.

The association between smoking and lung function is well-established and the central role of cigarette smoking has been carefully considered in the three projects. Although observational studies reported an association between low dietary antioxidant intake and decreased pulmonary function/higher risk of COPD, the majority of prior research was cross-sectional and there were no studies of elderly populations, a striking lack in the evidence base given that older populations face a greater risk of steep declines in lung function. Thus, the first project in this dissertation was a prospective cohort study of an elderly population. Much less evidence existed on the association between sequence variation in genes encoding

antioxidant-related enzymes and lung function. As such, before designing a study to investigate this association, a systematic overview was completed to evaluate the published studies investigating genetic factors influencing antioxidant capacity and COPD/COPD-related lung outcomes. The systematic overview guided study design and motivated gene selection for the third project, which investigated the association between sequence variants in antioxidant enzymes and lung function in an elderly cohort. The conclusions from these projects and their contribution to answering the overall research question are described below.

Dietary Antioxidants and Lung Function Decline: the Health Aging and Body Composition Study

In cigarette smokers a higher dietary intake of a broad range of nutrients with antioxidant properties was associated with a shallower rate of decline in lung function over 4 years of follow-up. Thus, the major finding from this longitudinal study of well-functioning older adults supports the hypothesis that dietary antioxidant intake is a modifiable factor that affects the rate of decline in FEV_1. Among current smokers, consumption of diets that were higher in vitamin C, vitamin E, β-carotene, and fruits and vegetables were associated with biologically meaningful differences in lung function trajectory compared to consuming diets low in these nutrients. Because the study collected dietary intake data at only one time during the follow-up of 9 years, the analysis explored whether findings were stronger when focusing on the time period surrounding this single measurement. The relation of dietary intake to lung function decline was strongest in participants who were current smokers at study baseline and had quit by their 4-year clinical visit: those participants with high intakes of vitamin E, β-carotene, vitamin C and fruit and vegetables had 83, 73, 52 and 32

ml/year lower rate of decline in FEV_1, respectively, compared to participants with low intake (high and low refer to the 90^{th} and 10^{th} percentile points, respectively, for the purposes of illustrating the associations, although underlying statistical models are continuous). All associations were diluted in models of antioxidant intake and 9-year rate of decline; a likely explanation for this dilution is that a single, early assessment of dietary intake was primarily informative of concurrent oxidant/antioxidant balance and proximal rate of decline.

The strong interactions seen between oxidant exposure and antioxidant intake provide support for the hypothesis that antioxidant capacity modifies the lung's response to smoke exposure. In the prospective cohort study potentially confounding variables were adjusted in statistical models, but efforts to fully adjust for all sources of confounding are ultimately imperfect, thus other approaches to address the research questions were pursued to strengthen the evidence relative to causal inference.

Genetic Variation and Gene Expression in Antioxidant-Related Enzymes and Risk of COPD: A Systematic Review

To address the limitations inherent in observational studies of antioxidant intake and lung function, the question of endogenous antioxidant capacity as a modifier of the association between smoke exposure and lung function was investigated with a genetic approach. Genetic factors, like dietary antioxidant intake, can affect the balance between oxidants and antioxidants in the lung, but studies of genetic variation are less likely to be affected by confounding bias. A systematic overview of prior studies of genes encoding antioxidant enzymes in relation to COPD and COPD-related lung phenotypes was conducted to understand the state of the field, to identify gaps in evidence, and to establish the baseline for a new study of genetic

variation in genes encoding antioxidant enzymes and COPD-related lung phenotypes. The systematic overview summarized studies of sequence variation and studies of gene expression to provide a more complete understanding of the full role of genetic factors in the pathophysiology of lung disease. At the time the systematic review was published, only one genome-wide association study had been conducted, and the majority of studies in the literature were candidate gene association studies, family-based studies, or micro-array (small sample) studies.

The systematic review included all studies of relevant lung outcomes and enzymes of known importance in maintaining redox balance in the lungs, including glutathione metabolism, the thioredoxin system, superoxide dismutases, and catalase. A total of 29 genetic association and 14 comparative gene expression studies met the inclusion criteria. There were very few instances where the evidence was sufficient to justify summary statements of effect for a particular gene, however the paper provided the first critical evaluation of research in this area, and identified targets for future studies.

Several methodological issues were highlighted by the systematic overview. Given the important role of smoking in COPD etiology and intraindividual variation in the response to smoke exposure, careful consideration of smoking parameters during study design and analysis are vital to uncovering gene-disease associations. Gene-smoking interactions were rarely tested in published papers (although analyses were sometimes limited to smoke-exposed participants) and a description of the smoking status of participants was occasionally omitted. The systematic overview also revealed that a limited number of genes, and SNPs within those genes, have been evaluated for an association with disease risk, an important finding considering the broad, interrelated network of antioxidant enzymes in the lungs. For instance, 24 published association studies studied genes encoding glutathione s-Transferases

(GSTs), but no association studies studied genes associated with thioredoxin metabolism. Furthermore, in the association studies studying GSTs, only 3 genes (out of 18 total GST genes) and 4 variants were evaluated, comprising a limited study of the gene family. The limited consideration of environmental interactions and the scarcity of research on all but a few SNPs in a handful of genes, there were serious limitations to any inferences about the role of sequence variation in antioxidant enzymes in relation to disease risk.

Genetic Variation in Antioxidant Enzymes and Lung Function in the Health, Aging, and Body Composition (Health ABC) Study

With the conclusions from the systematic review in hand, a new study was designed to investigate whether the role of genetic variation in antioxidant enzymes in lung phenotypes. Two spirometry parameters were evaluated for these analyses: the ratio between the forced expiratory volume in the 1st second and the forced vital capacity (FVC) (thus, the FEV_1/FVC), and the percent achieved of the FEV_1 value that was predicted for that participant based on height, race, gender, and age (% predicted FEV_1). The ratio and the % predicted FEV_1 are used to diagnose and stage COPD. The ratio identifies decreases in FEV_1 in relation to FVC, which is a hallmark of airflow obstruction. The % predicted FEV_1 is used in conjunction with the FEV_1/FVC ratio as a method of grading severity of airflow obstruction. The quantitative markers of lung phenotype were preferred over a disease-based approach for several reasons: quantitative measures of lung phenotype increased the power to detect associations, variability in these outcomes may have functional significance in an elderly population beyond COPD diagnosis, analyses with these measures may be more robust than analyses of COPD diagnosis given debates among experts regarding the most

appropriate thresholds for diagnosis, and, importantly, these measures are available at high quality for a large proportion of the Health, Aging, and Body Composition cohort. As these measures underlie COPD diagnosis, they are considered as robust predictors of disease risk, and indeed in elderly populations spirometry also predicts all-cause mortality(1).

To address a gap in the current literature, this study was designed to evaluate the entire network of enzymes of known importance in redox balance that are expressed in the lung. Fifty-six genes were selected, the majority of which had never been considered in an association study of COPD-related lung outcomes, but, given their function, may have an important role in determining an individual's response to the oxidative assault of cigarette smoking. As the interest was in identifying genes of importance in disease risk, markers were selected to fully represent the genes and their regulatory regions. Although preference was given to inclusion of SNPs that had previously been investigated or with known function in relation to these outcomes, the evidence base was such that this constituted only 2% of SNPs selected.

The results from these analyses are novel: of the genes represented among the most statistically significant SNPs, only 1 gene had been previously evaluated in an association study (*GCLC*), suggesting the benefits of taking a network approach. A large proportion of the genes (64%) represented in the most statistically significant results produce mitochondrial enzymes, suggesting new hypotheses about the importance of this cellular compartment in lung function phenotypes and COPD risk.

Currently, researchers are turning to genome-wide association studies (GWAs) to discover novel gene regions of interest in relation to a particular phenotype. The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium recently completed a meta-analysis of GWAS of FEV_1 and FEV_1/FVC in

over 20,000 participants in 4 epidemiologic cohort studies, including Atherosclerosis Risk in Communities, Cardiovascular Health Study, Framingham Heart Study, and the Rotterdam Study(2). Comparisons between the Health ABC results and the CHARGE results are of interest. None of the genes in our most statistically significant results list (nor any of our studied genes) appeared in the top 2000 hits in the CHARGE study. According to a recent study, it is unlikely that this discrepancy is a result of the failure of the GWAs to appropriately capture the genetic variation present in our studied genes (3). Although a replication of the Health ABC findings by the CHARGE cohorts would strengthen the finding, an absence of replication may be explained by several scenarios. The CHARGE GWAS considered a linear, additive coding of SNPs, such that 2 copies of an allele were expected to have an effect size that was approximately double the effect of a single copy of the allele, and a single parameter estimate was used to describe this relationship (thus, regression coefficients and p values test this additive effect). In the Health ABC analyses, the best coding for each SNP (on the basis of strength of association with outcome) was used, and additive coding was modeled as a set of dummy variables to avoid assumptions about linearity in the genotype—phenotype association. Given that SNPs are known to affect disease risk in a variety of ways (i.e. dominant, recessive, heterozygote advantage, additive/multiplicative), the lack of replication may be explained by a difference in coding between CHARGE and Health ABC. Although the CHARGE GWAS stratified by smoke exposure (with never smokers analyzed separately from current and former smokers), there was no formal test of gene-environment interactions (for instance, with a product term between the SNP and smoking parameter).

When the models from the 35 most statistically significant results in the Health ABC analyses were rerun using the coding and stratification strategies described in the CHARGE GWAS, only 2 of the 35 SNPs remained associated with the outcome at the

level of statistical significance used to define the set of top hits ($p<0.002$). Clearly, decisions regarding coding of alleles and modeling of gene-environment interactions are of vital importance in genetic association studies.

Another notable difference between the Health ABC study and the CHARGE GWAS meta-analysis is the demography of the populations studied. The Health ABC population was about 40% Black, while all of the participants in the CHARGE analyses were of European ancestry (an important difference considering the proportionately higher number of findings among African Americans in the Health ABC results). Also, the mean age of the participants in the CHARGE GWAS was considerably lower (58 vs 73 years) and, consistently, the mean FEV_1 was higher (2,807 vs 2,215 ml); a smaller proportion of these populations may be experiencing the age-related increases in susceptibility to low lung function. Population characteristics are also of vital importance in drawing comparisons between genetic epidemiology studies.

Significance of This Work

This dissertation was designed to address the susceptibility hypothesis, that is the hypothesis that COPD risk varies among cigarette smokers. Understanding the determinants of susceptibility would enable identification of those at greatest risk of adverse outcomes and assist in targeting appropriate therapies to this population. Based on a number of observations in COPD epidemiology, this work investigates the hypothesis that variability in antioxidant defenses contribute to the variability in smoke exposure response observed in COPD risk. Through explorations of both dietary and genetic sources of variation in antioxidant defenses, evidence has been provided in support of a role of antioxidant defenses in determining disease susceptibility.

There are implications of this work beyond the scope of this particular research question, as well. First, the dietary antioxidant—FEV_1 decline paper provides key observations for the modeling of lung function decline. While the tendency may be to include as many measurements of lung function as possible in order to have the most complete picture of lung function trajectory, careful consideration must be given to the timing of exposure measurements relative to the interval of decline for which those measurements are most appropriate. For instance, there were statistically significant differences in the antioxidant—lung function decline association between participants whose oxidant burden changed during 4 years of follow-up and those without change (quitters vs. those who continued to smoke): having the most concurrent measures of oxidant burden was important in uncovering the antioxidant—lung function decline association in these participants. Similarly, dietary antioxidant measured at the first year of follow-up appeared to be far more relevant to the earliest interval of decline (from baseline to the 4^{th} year of follow-up) than to more distant intervals (including the 7^{th} and 9^{th} years of follow-up); an observation that is consistent with the changes in diet that are likely to occur during this life stage.

Additional broad implications of this dissertation are provided by the antioxidant enzyme—lung function project. In an era of research dominated by GWAS, there is still relevance in this hypothesis-oriented approach. The biological hypotheses at the core of this work motivate design and analysis that incorporates factors of relevance to the biological model; the interaction between genes and smoking was central to the biological model, so every effort was made to effectively model this interaction within our analyses. In contrast, the GWAS study on a similar question is not directly motivated by a biological model and gene-environment interactions were not considered, potentially masking the effects that would have been observed if the analysis were specifically looking at that question.

Additionally, the discordance between the results by race deserve consideration. In the SNPs project, there were proportionately more statistically significant results in African Americans, which may have been the end result of a greater burden of current smoking, a lower mean pulmonary function on average, or variability in genetic sequence. Disentangling this story is important, and has implications for other phenotypes as well. These results suggest that not only are the conclusions from European Americans not directly transferable to other populations, but that variations in exposure to environmental and genetic factors may make study within African Americans a more informative design for discovery approaches for certain phenotypes. Comparison of African Americans with Africans, who have similar genetic variation but different environmental exposures, may provide further insight into the extent to which these apparent racial differences may be the result of increased oxidative burden. Similarly, a study conducted in European Americans with a greater proportion of current cigarette smokers may yield results more concordant with those currently observed in African Americans, providing support for the hypothesis that these differences are driven by proportion of participants under smoking-induced oxidative stress. Continuing work on the African-American genome and disease associations from centers such as the National Human Genome Center (Howard University) and the Center for Research on Genomics and Global Health (National Human Genome Research Institute) will certainly offer further insight into this question.

Future Directions

While the work comprising this dissertation provides important evidence regarding the role of antioxidant defenses in COPD risk, it also suggests future

directions for solidifying the understanding of this relation, particularly in regard to the most novel project conducted in this research, the association study between genetic variation in antioxidant enzymes and lung function. Further work should be conducted within this population to consider haplotypes in the genes studied, and to consider different lung outcomes of importance in COPD risk, particularly rate of decline in FEV_1, as lung function trajectory may isolate those who are particularly susceptible to adverse outcomes. Also, other methods of investigating population substructure beyond self-reported race should be explored to further our understanding of the differences observed in these analyses. Specifically, principal components analyses to identify meaningful subgroups of variability within participants and the use of admixture markers to represent degree of "African-ness" of African American participants would be useful methods of addressing the heterogeneity expected within groups defined imprecisely by race. Additionally, the interrelatedness of the enzymes and nutrients suggest several further avenues for more direct investigation of gene-nutrient interactions and gene-gene interactions. Interaction between SNPs is biologically plausible given that many of these enzymes are linked in a pathway (i.e., with one enzyme providing a substrate/precursor for another, or enzymes sharing a common substrate). Such an investigation would highlight not only genes, but pathways that are of particular relevance to reducing disease risk. There is already evidence supporting interactions between some of these enzymes and dietary antioxidants: vitamin C can reduce glutathione from its oxidized disulfide conformation to the form with antioxidant activity (potentially affecting a whole range of glutathione-associated enzymes)(4); and, there is evidence for modification in expression and/or activity of selenoproteins with decreased selenium intake (5-9). Evidence of interactions between diet and SNPs in antioxidant enzymes would provide further understanding of etiology, but, importantly, would also suggest dietary

antioxidants as a simple preventative intervention.

Several of the genes highlighted in the antioxidant enzyme—lung function project have been considered minimally, if at all, as potential contributors to COPD susceptibility. As such, further work at a basic science level is necessary to understand how these enzymes respond to oxidative insult. Of particular importance is the preponderance of mitochondrial enzymes identified among the most statistically significant results. Little work has been conducted to investigate the potential importance of the mitochondria in susceptibility to COPD, although there is some evidence of a role for the mitochondria in the excessive production of oxidative stress observed with cigarette smoke exposure. This relation needs further investigation.

As observational studies of dietary antioxidants and lung outcomes are for the most part positive across a broad range of exposure and outcome definitions, further work to investigate this relation should follow a different strategy to strengthen causal inference. Specifically, one unanswered question is how increased antioxidant intake affects the lung tissue that is the site of the disease process. Although the relation of antioxidant supplementation and systemic levels of antioxidants is well-established, there is little known about the effects of systemic supplementation on antioxidant levels in the relevant lung compartments: the epithelial lining fluid and associated cells, and in epithelial cells. These dietary antioxidants may also exert an effect on expression of antioxidant enzymes, particularly those regulated with an antioxidant response element. A pilot study of short-term antioxidant supplementation on smokers and subsequent changes in antioxidant concentration in the epithelial lining fluid and changes in gene expression is currently underway to investigate these questions.

In summary, evidence generated in this dissertation supports the hypothesis

that variability in antioxidant defenses contributes to the variability in risk of COPD observed among cigarette smokers. While the specific mechanisms by which dietary antioxidants and antioxidant enzymes influence the lung function parameters associated with COPD risk remain to be elucidated, this work has contributed to the evidence base and has highlighted a number of questions for future research, particularly as related to the findings of the genetic association study. Based on the current evidence regarding the association between determinants of antioxidant defenses and lung outcomes, antioxidant strategies may offer a promising opportunity for the prevention of this prevalent and increasingly prominent disease.

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APPENDIX

SUPPLEMENT A.1

SEARCH STRATEGY AND RESULTS

GENETIC VARIATION AND GENE EXPRESSION IN ANTIOXIDANT-RELATED ENZYMES AND RISK OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A SYSTEMATIC REVIEW

Searches of PubMed were conducted using the keywords "antioxidant" [AND] "gene" in combination with "COPD", "emphysema", and "chronic bronchitis." Searches also considered disease keywords along with each of the enzymes of interest. Searches were performed up to August 2007. Our interest was in enzymes and enzyme networks with direct antioxidant function, so the wide range of metal-related enzymes (e.g. heme oxygenase), which influence redox status indirectly by altering metal concentrations, were not considered. Studies of systemic expression were not included as the relation between these values and expression levels in pulmonary cells and tissue is not well defined, making the contribution of these studies to the review questions unclear. Search results were supplemented by reviewing reference lists of identified papers. Only original research was considered. Searches identified 223 articles for consideration. Exclusions were made for the following reasons: not original research (57); not on adult humans (56); not genes of interest (9); outcome not disease or lung function (in association studies: 2); exposure not disease or smoking (in expression studies: 32); expression studies of cells or tissues that were not lung-specific (23); not an association or expression study (7). After reviewing reference lists, an additional 7 studies were added, for a total of 29 genetic association studies and 14 studies evaluating differential expression of antioxidant genes in lung tissue. All studies considered are summarised briefly in Table A.2.

TABLE A.2

CHARACTERISTICS OF INCLUDED STUDIES
GENETIC VARIATION AND GENE EXPRESSION IN ANTIOXIDANT-RELATED ENZYMES AND RISK OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A SYSTEMATIC REVIEW

Association Studies

Ref	First Author, Pub Year	Gene	Populations Compared	Outcome of Interest
1	Hu, 2006	GCLM	Chinese COPD patients and asymptomatic smokers	COPD
2	Liu, 2007	GCLC	Chinese COPD patients and controls (matched gender, age, smoking)	COPD
3	Cheng, 2004	GSTM1 GSTP1 GSTT1	Taiwanese COPD patients and asymptomatic smokers	COPD
4	Yanchina, 2007	GSTM1	Russian male COPD patients and asymptomatic smokers	COPD
5	Dialyna, 2003	GSTM1	Greek lung cancer patients with COPD and with pulmonary fibrosis or tuberculosis (both groups include smokers and nonsmokers)	COPD
6	Hersh, 2005	GSTM1 GSTP1	White American emphysema patients (smokers and nonsmokers) and asymptomatic ever smokers	Emphysema
7	Harrison, 1997	GSTM1	Scottish lung cancer patients with emphysema (smokers), with lung cancer only (smokers), and healthy blood donors (unknown smoking status)	Emphysema
8	Budhi, 2003	GSTM1 GSTP1 GSTT1	Japanese male heavy smokers with and without emphysematous changes	Emphysematous changes
9	Baranov, 1996	GSTM1	French chronic bronchitis patients and asymptomatic individuals	Chronic Bronchitis
10	Baranova, 1997	GSTM1	French smokers: chronic bronchitis, asymptomatic	Chronic Bronchitis
11	Imboden, 2007	GSTM1 GSTP1 GSTT1	Predominantly European Caucasian general population (smokers and nonsmokers)	Rate of decline: FEV_1, FVC, FEF_{25-75}
12	Tkacova, 2004	GSTM1 GSTT1	Slovakian smokers with lung cancer	FEV_1 % predicted, FVC % predicted
13	Calikoglu, 2006	GSTM1 GSTP1 GSTT1	Turkish male COPD patients and controls (matched on age and smoking status)	COPD
14	Ishii, 1999	GSTP1	Japanese COPD patients and asymptomatic smokers	COPD
15	Vibhuti, 2007	GSTP1	Indian COPD patients and asymptomatic smokers	COPD, FEV_1 % predicted
16	He, 2004	GSTM1 GSTP1 GSTT1	White North American smokers	Highest vs. lowest quintile of FEV_1 % predicted

TABLE A.2 (continued)

Ref	First Author, Pub Year	Gene	Populations Compared	Outcome of Interest
17	Hersh, 2006	GSTM1 GSTP1 mGST1	White American emphysema patients	FEV1 % predicted
18	He, 2002	GSTM1 GSTP1 GSTT1	White North American smokers	Risk of steepest vs. slowest quintile of rate of FEV_1 decline
19	Mak, 2007	SOD2 CAT	Chinese COPD patients and asymptomatic ever smokers	COPD
20	Juul, 2006	SOD3	Danish COPD patients and asymptomatic individuals	COPD, incidence of COPD hospitalization, FEV_1 % predicted, FVC % predicted, FEV_1/FVC
21	Young, 2006	SOD1 SOD2 SOD3 CAT	COPD patients, asymptomatic smokers, and healthy blood donors (all of European descent)	COPD
22	Chan-Yeung, 2007	GSTM1 GSTP1 GSTT1	Chinese COPD patients and controls (matched on age and smoking history)	COPD
23	Korytina, 2004	GSTM1 GSTP1	Russian COPD patients and asymptomatic individuals	COPD
24	Lu, 2002	GSTP1	Chinese male COPD patients and asymptomatic smokers	COPD
25	Rodriguez, 2005	GSTP1	Spanish COPD patients and asymptomatic individuals	COPD
26	Yim, 2002	GSTP1	Korean COPD patients (smokers and nonsmokers) and asymptomatic smokers	COPD
27	Harries, 1997	GSTP1	Scottish Caucasian COPD patients and healthy individuals	COPD
28	Xiao, 2004	GSTP1	Chinese COPD patients and asymptomatic individuals	COPD
29	Yim, 2000	GSTM1 GSTT1	Korean COPD patients (smokers and nonsmokers) and asymptomatic smokers	COPD

TABLE A.2 (continued)

Expression Studies

Ref	First Author, Pub Year	Gene	Populations Compared	Tissue Type(s)	Assay Method(s)
30	Rahman 2000	GCLC	COPD patients and asymptomatic ever smokers (all undergoing lung resection for cancer)	Alveolar epithelium, airway epithelial cells	In situ hybridisation
31	Lin 2005	GCLC	COPD patients and patients without COPD (all undergoing lung resection for tumor)	Alveoli, bronchi, inflammatory cells	In situ hybridization, Immunohistochemistry
32	Harju 2002	GCLC GCLM	COPD patients (smokers), asymptomatic smokers, and asymptomatic nonsmokers (all undergoing lung resection for tumor)	Bronchioli epithelium, central bronchus epithelium, alveolar macrophages	Immunohistochemistry
33	Neurohr 2003	GCLC GCLM	Asymptomatic smokers and nonsmokers	Alveolar macrophages	RT-PCR
34	Pierrou 2007	Many	COPD patients at various stages, asymptomatic smokers, and nonsmokers	Bronchial epithelial cells	Microarray
35	Hackett 2003	Many	Asymptomatic smokers and nonsmokers	Airway epithelial cells	Microarray
36	Tomaki 2007	Many	COPD patients, smokers, and nonsmokers (all undergoing lung resection for cancer)	Peripheral lung tissue	RT-PCR
37	Comhair 2000	GPX3	Asymptomatic smokers and nonsmokers	Airway epithelial cells, alveolar macrophages	Northern blot
38	Peltoniemi 2006	GLRX	COPD patients at various stages and smokers without COPD (all undergoing lung resection for tumor or lung transplantation for advanced COPD)	Lung tissue homogenate, sputum supernatants	Western blot
39	Heguy 2006	G6PD	Asymptomatic smokers and nonsmokers	Alveolar macrophages	Microarray
40	Harju 2007	GSTO1	COPD patients at various stages, smokers without COPD, and nonsmokers (all undergoing lung resection for tumor or lung transplantation for advanced COPD)	Alveolar epithelium, bronchial epithelium, lung tissue homogenate, sputum, alveolar macrophages, pulmonary blood vessels	Immunohistochemistry, Western blot

TABLE A.2 (continued)

Ref	First Author, Pub Year	Gene	Populations Compared	Tissue Type(s)	Assay Method(s)
[41]	Yigla 2007	SOD	Smokers with COPD, nonsmokers with COPD, asymptomatic smokers, and asymptomatic nonsmokers	Bronchoalveolar lavage fluid	Enzyme assay
[42]	Harju 2004	SOD1 SOD2 SOD3	Smokers (with COPD GOLD stage 0-2) and nonsmokers; Smokers with COPD, asymptomatic smokers, and asymptomatic nonsmokers (all undergoing lung resection for tumor)	Lung tissue homogenate, central bronchus epithelium, bronchioli epithelium, alveolar epithelium, alveolar macrophages, pulmonary blood vessels	Western blot, immunohistochemistry
[43]	Kondo 1994	SOD1	Asymptomatic elderly male smokers and nonsmokers	Alveolar macrophages	Northern blot

*reference number linked to bibliography (Supplement A.5)

TABLE A.3

GENETIC ASSOCIATION STUDIES OF GENES RELATED TO ANTIOXIDANT FUNCTION IN PATIENTS WITH COPD IN COMPARISON TO CONTROLS

GENETIC VARIATION AND GENE EXPRESSION IN ANTIOXIDANT-RELATED ENZYMES AND RISK OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A SYSTEMATIC REVIEW

Most association studies reported odds ratios and 95% confidence intervals. When odds ratios were not given or used the variant genotype as the reference group, these measures were recalculated by the authors. Confounder-adjusted models were used when available. Quality of studies was evaluated in four categories of study features: population selection, exposure assessment, outcome assessment, and handling of covariates. Each study was scored on whether the presented methods did ("+") or did not ("-") appropriately address potential bias in that category ("+"), or whether risk of bias could not be determined from given information ("0"). The detailed results across all studies are presented by gene in functional groups, and studies are repeated in the table if they included data on more than one gene or variant. Association studies are sorted within groups of genes by the outcome or group considered; for example, COPD and related diagnoses are considered before quantitative traits.

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
Glutathione Synthesis						
GCLC C → T at -129 Results in decreased expression [44] *rs17883901*						
Liu 2007	Han Chinese	C/C	COPD patients (166)	41.6	*ref*	+0++
			Healthy controls (166)	48.2		
		C/T	COPD patients (166)	58.4	1.83 (1.00, 3.36)	
			Healthy controls (166)	51.8		
GCLM C → T at -588 Results in decreased plasma glutathione [45] *rs41303970*						
Hu 2006	Chinese	C allele	COPD patients (104)	79.2	*ref*	+0+-
			Asymptomatic smokers (124)	91.9		
		T allele	COPD patients (104)	20.8	3.0 (1.7, 5.3)	
			Asymptomatic smokers (124)	8.1		
Glutathione Antioxidant Activity and Recycling						
(No studies)						
Glutathione Conjugation and Export						
GSTM1 NULL Homozygous deletion resulting in complete lack of activity [46]						
Calikoglu 2006	Turkish men	Null	Stable COPD patients (149)	48.6	1.38 (0.87, 2.19)	+0++
			Healthy controls (150)	40.7	*ref*	
Chan-Yeung 2007	Chinese	Null	COPD patients (163)	60.1	1.16 (0.74, 1.80)	+0++
			Healthy controls (163)	56.5	*ref*	
Cheng 2004	Taiwanese	Null	COPD patients (184)	61.4	2.2 (1.3, 3.5)	+0++
			Asymptomatic controls (212)	42.5	*ref*	
Korytina 2004	Russian	Null	COPD patients (104)	50.5	1.37 (0.83, 2.25)	-00-
			Asymptomatic controls (164)	42.7	*ref*	

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
Yanchina 2004	Russian men	Null	COPD patients (72)	38.9	2.47 (1.10, 5.54)	00--
			Asymptomatic smokers (39)	20.5	*ref*	
Yim 2000	Korean	Null	COPD patients (83)	57.0	0.72 (0.38, 1.36)	-0+-
			Asymptomatic smokers (76)	65.0	*ref*	
Dialyna 2003	Greek lung cancer patients	Null	COPD patients (includes non-smokers)(21)	66.7	*8.0 (1.32, 48.18)*[ix]	-0--
			Patients with a history of pulmonary fibrosis or tuberculosis (includes non-smokers)(10)	20.0	*ref*	
Hersh 2005	White American	Null	Emphysema patients (includes non-smokers) (304)	58.0	1.17 (0.87, 1.57)	+0++
			Asymptomatic males with smoking history (441)	54.0	*ref*	
Harrison 1997	Scottish lung cancer patients undergoing resection surgery	Null	Patients with Emphysema (111)	65.0	1.78 (0.93, 3.41)[iv] 1.61 (1.04, 2.50)[v]	-++0
			Patients with lung cancer only (57)	51.0	*ref*	
			Healthy blood donors (384)	53.0	*ref*	
Baranov 1996	French	Null	Severe Chronic Bronchitis (53)	73.6	3.47 (1.68, 7.16)	-00-
			Mild Chronic Bronchitis (22)	40.9	0.86 (0.34, 2.20)	
			No Chronic Bronchitis (101)	44.6	*ref*	
Baranova 1997	French	Null	Severe Chronic Bronchitis (87)	71.3	3.01 (1.74, 5.21)	++++
			Moderate Chronic Bronchitis (102)	65.7	2.15 (1.30, 3.57)	
			No Chronic Bronchitis (172)	47.1	*ref*	
Budhi 2003	Japanese	Null	Heavy smokers with emphysematous changes (63)	57.1	1.1 (0.6, 2.0)	00++
			Heavy smokers without emphysematous changes (172)	54.1	*ref*	
He 2002	Predominantly White North American	Null	Smokers with steepest decline in FEV_1 (299)	47.4	0.95 (0.6, 1.35)	++++
			Smokers with slowest decline in FEV_1 (322)	50.2	*ref*	

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
Imboden 2007	European Caucasians (predominantly)	Null	Linear regression of genotype on FEV_1 ml decline/yr (4686)	52.9	Men: -2.1(-4.5,0.3) [viii] Women: 0.5(-1.2,2.2)	++++
He 2004	White North American	Null	Smokers with lowest FEV_1 % predicted (544)	54.0	1.09 (0.83, 1.42)	++++
			Smokers with highest FEV_1 % predicted (544)	54.3	*ref*	
Hersh 2006	White American emphysema patients	Null	Linear regression of genotype on FEV_1 % predicted (304)	NA	p> 0.10	+0++
Tkacova 2004	Slovakian lung cancer patients	Null	GSTM1 Null: Mean FEV_1 % predicted = 75.8 ± 2.5 (110)	49.0	p<0.02	-+++
			GSTM1 Non-null: Mean FEV_1 % predicted = 86.6 ± 3.6 (110)	51.0		
Imboden 2007	European Caucasians (predominantly)	Null	Linear regression of genotype on FVC ml decline/yr (4686)	52.9	Men: -0.8(-4.0,2.5) [viii] Women: 1.1(-1.2,3.4)	++++
Tkacova 2004	Slovakian lung cancer patients	Null	GSTM1 Null: Mean FVC % predicted = 83.9 ± 2.1 (110)	49.0	p<0.01	-+++
			GSTM1 Non-null: Mean FVC % predicted = 90.3 ± 2.0 (110)	51.0		
Imboden 2007	European Caucasians (predominantly)	Null	Linear regression of genotype on FEF_{25-75} ml decline//yr (4686)	52.9	Men: -4.1(-9.2,0.9) [viii] Women:-0.8(-4.7,3.1)	++++

GSTP1 Ile105Val A→G at nucleotide 313, changes affinity for specific substrates: valine substitution increased detoxification activity of the enzyme when challenged with polycyclic aromatic hydrocarbons and decreased activity is observed with other substrates (e.g. 1-chloro-2,4-dinitrobenzene). [47, 48] Val substitution has higher heat stability than Ile [49] *rs1695*

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
Calikoglu 2006	Turkish men	A/A	Stable COPD patients (149)	61.1	*ref*	+0++
			Healthy controls (150)	38.0		
		A/G	Stable COPD patients (149)	29.2	0.47 (0.28, 0.80)	
			Healthy controls (150)	38.0		
		G/G	Stable COPD patients (149)	9.7	0.25 (0.12, 0.50)	
			Healthy controls (150)	24.0		
Chan-Yeung 2007	Chinese	A/A	COPD patients (163)	68.7	*ref*	+0++
			Healthy controls (163)	69.6		
		A/G	COPD patients (163)	26.4	0.91 (0.56, 1.49)	
			Healthy controls (163)	29.2		
		G/G	COPD patients (163)	4.9	*4.0 (0.83, 19.25)*[ix]	
			Healthy controls (163)	1.2		
Cheng 2004	Taiwanese	A/A	COPD patients (184)	52.7	*ref*	+0++
			Asymptomatic controls (212)	46.7		
		A/G	COPD patients (184)	42.4	0.81 (0.54, 1.22)	
			Asymptomatic controls (212)	46.2		
		G/G	COPD patients (184)	4.9	0.61 (0.26, 1.47)	
			Asymptomatic controls (212)	7.1		
Harries 1997	Caucasians (Scottish)	A/A	COPD patients (79)	43.0	*ref*	-0--
			Healthy individuals (155)	51.0		
		A/G	COPD patients (79)	44.3	1.23 (0.69, 2.19)	
			Healthy individuals (155)	42.5		
		G/G	COPD patients (79)	12.7	2.32 (0.89, 6.09)	

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
			Healthy individuals (155)	6.5		
Ishii 1999	Japanese	A/A	COPD patients (53)	79.0	*ref*	+0+-
			Asymptomatic smokers (50)	52.0		
		A/G	COPD patients (53)	21.0	0.31 (0.13, 0.74)	
			Asymptomatic smokers (50)	44.0		
		G/G	COPD patients (53)	0.0	NA	
			Asymptomatic smokers (50)	4.0		
Korytina 2004	Russian	A/A	COPD patients (104)	59.1	*ref*	-00-
			Asymptomatic controls (164)	63.2		
		A/G	COPD patients (104)	33.7	1.07 (0.62, 1.83)	
			Asymptomatic controls (164)	33.5		
		G/G	COPD patients (104)	7.2	2.29 (0.69, 7.53)	
			Asymptomatic controls (164)	3.3		
Lu 2002	Chinese men	A/A	COPD patients (97)	72.0	*ref*	+0++
			Asymptomatic smokers (67)	61.0		
		A/G	COPD patients (97)	23.0	0.54 (0.27, 1.08)	
			Asymptomatic smokers (67)	36.0		
		G/G	COPD patients (97)	5.0	*1.46 (0.27, 7.89)*[ix]	
			Asymptomatic smokers (67)	3.0		
Rodriguez 2005	Spanish	A/A	COPD patients (99)	53.1	*ref*	-+++
			Asymptomatic smokers and nonsmokers (198)	49.0		
		A/G	COPD patients (99)	36.7	0.76 (0.46, 1.28)	
			Asymptomatic smokers and nonsmokers (198)	44.0		
		G/G	COPD patients (99)	10.2	1.43 (0.59, 3.50)	

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
			Asymptomatic smokers and nonsmokers (198)	6.6		
Vibhuti 2007	Indian smokers	A/A	COPD patients (202)	52.0	*ref*	00++
			Healthy individuals (136)	66.2		
		A/G	COPD patients (202)	37.1	1.53 (0.96, 2.45)	
			Healthy individuals (136)	30.9		
		G/G	COPD patients (202)	10.9	*4.71 (1.57, 14.2)* [ix]	
			Healthy individuals (136)	2.9		
Xiao 2004	Chinese	A/A	COPD patients (includes nonsmokers)(100)	57.0	*ref*	-0++
			Asymptomatic smokers and nonsmokers (100)	70.0		
		A/G	COPD patients (includes nonsmokers) (100)	40.0	1.69 (0.94, 3.06)	
			Asymptomatic smokers and nonsmokers (100)	29.0		
		G/G	COPD patients (includes nonsmokers) (100)	3.0	*3.68 (0.37, 36.38)* [ix]	
			Asymptomatic smokers and nonsmokers (100)	1.0		
Yim 2002	Korean	A/A	COPD patients (includes never-smokers) (89)	71.0	*ref*	-0+-
			Asymptomatic smokers (94)	61.0		
		A/G	COPD patients (includes never-smokers) (89)	27.0	0.62 (0.33, 1.17)	
			Asymptomatic smokers (94)	37.0		
		G/G	COPD patients (includes never-smokers) (89)	2.0	*0.90 (0.12, 6.64)* [ix]	
			Asymptomatic smokers (94)	2.0 2.0		
Hersh 2005[vi]	White American	A allele	Emphysema patients (includes nonsmokers) (304)	65.0	*ref*	+0++
			Asymptomatic males with smoking history (441)	70.0		
		G allele	Emphysema patients (includes nonsmokers) (304)	35.0	1.25 (0.92, 1.71)	

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
			Asymptomatic males with smoking history (441)	30.0		
Budhi 2003	Japanese	A/A	Heavy smokers with emphysematous changes (63)	77.8	*ref*	00++
			Heavy smokers without emphysematous changes (172)	73.3		
		A/G	Heavy smokers with emphysematous changes (63)	20.6	0.78 (0.39, 1.57)	
			Heavy smokers without emphysematous changes (172)	25.0		
		G/G	Heavy smokers with emphysematous changes (63)	1.6	*0.86 (0.09, 8.44)*[ix]	
			Heavy smokers without emphysematous changes (172)	1.7		
He 2002	Predominantly White North American	A/A	Smokers with steepest decline in FEV_1 (299)	40.0	*ref*	++++
			Smokers with slowest decline in FEV_1 (322)	39.0		
		A/G	Smokers with steepest decline in FEV_1 (299)	48.6	0.92 (0.66, 1.31)	
			Smokers with slowest decline in FEV_1 (322)	51.2		
		G/G	Smokers with steepest decline in FEV_1 (299)	11.4	1.12 (0.64, 1.97)	
			Smokers with slowest decline in FEV_1 (322)	9.8		
He 2004	White North American	A/A	Smokers with lowest FEV_1 % predicted (544)	44.7	*ref*	++++
			Smokers with highest FEV_1 % predicted (544)	44.7		
		A/G	Smokers with lowest FEV_1 % predicted (544)	42.1	0.65 (0.43, 0.97)	
			Smokers with highest FEV_1 % predicted (544)	46.0		
		G/G	Smokers with lowest FEV_1 % predicted (544)	13.2	2.01 (1.14, 3.59)	
			Smokers with highest FEV_1 % predicted (544)	9.3		
Vibhuti 2007	Indian smokers	A/A	COPD patients: FEV_1 % predicted 61.9 ± 29.6 L (105)	52.0	$p > 0.05$	00++
		A/G & G/G	COPD patients: FEV_1 % predicted 57.7 ± 24.1 L (97)	48.0		
Vibhuti 2007	Indian smokers	A/A	Asymptomatic smokers: FEV_1 % predicted 110.7 ± 22.4 L (90)	66.2	$p > 0.05$	00++

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
		A/G & G/G	Asymptomatic smokers: FEV_1 % predicted 109.1 ± 16.7 L (46)	33.8		
Imboden 2007	European Caucasians (predominantly)	A/G	Linear regression of genotype on FEV_1 ml decline/yr (4686)	43.2	Men:0.09(-2.4,2.6) [viii] Women:-0.6(-2.4,1.2)	++++
Imboden 2007	European Caucasians (predominantly)	G/G	Linear regression of genotype on FEV_1 ml decline/yr (4686)	9.4	Men: -2.5(-6.6,1.7) [viii] Women:-0.2(-3.3,2.9)	++++
Imboden 2007	European Caucasians (predominantly)	A/G	Linear regression of genotype on FVC ml decline/yr (4686)	43.2	Men: -0.1(-3.5,3.3) [viii] Women: 0.6(-1.8,3.0)	++++
Imboden 2007	European Caucasians (predominantly)	G/G	Linear regression of genotype on FVC ml decline/yr (4686)	9.4	Men: -3.2(-9.0,2.5) [viii] Women: 1.5(-2.6,5.6)	++++
Imboden 2007	European Caucasians (predominantly)	A/G	Linear regression of genotype on FEF_{25-75} ml decline/yr (4686)	43.2	Men: -2.7(-8.0,2.6) [viii] Women:-3.0(-7.0,1.1)	++++
Imboden 2007	European Caucasians (predominantly)	G/G	Linear regression of genotype on FEF_{25-75} ml decline/yr (4686)	9.4	Men:-7.3(-16.1, 1.6)[viii] Women: 0.5(-6.5,7.5)	++++
Hersh 2006	White American emphysema patients		Linear regression of an additive genetic model on FEV_1 % predicted (304)	NA	p> 0.10	+0++
GSTP1 Ala114Val C→T at nucleotide 341, changes Ala to Val at 114 [50] *rs1138272*						
Ishii 1999	Japanese	C/C	COPD patients (53)	100	Could not be calculated	+0+-
			Asymptomatic smokers (50)	100		
Vibhuti 2007	Indian smokers	C/C	COPD patients (202)	60.9	*Ref*	00++
			Healthy individuals (136)	77.9		
		C/T	COPD patients (202)	28.2	1.56 (1.08, 2.70)	

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
			Healthy individuals (136)	17.6		
		T/T	COPD patients (202)	10.9	2.47 (1.15, 6.12)	
			Healthy individuals (136)	4.4		
Hersh 2005[vi]	White American	C allele	Emphysema patients (includes nonsmokers) (304)	92.0	*Ref*	+0++
			Asymptomatic males with smoking history (441)	91.0		
		T allele	Emphysema patients (includes nonsmokers) (304)	8.0	0.86 (0.51, 1.46)	
			Asymptomatic males with smoking history (441)	9.0		
Hersh 2006	White American emphysema patients		Linear regression of additive genetic model on FEV_1 % predicted in emphysema patients (304)	NA	p> 0.10	+0++
Vibhuti 2007	Indian smokers	C/C	COPD patients: FEV_1 % predicted 63.9 ± 28.5 L (123)	60.9	p < 0.05	00++
		C/T & T/T	COPD patients: FEV_1 % predicted 56.2 ± 22.4 L (79)	39.1		
Vibhuti 2007	Indian smokers	C/C	Asymptomatic smokers: FEV_1 % predicted 110.1 ± 21.2 L (106)	77.9	p > 0.05	00++
		C/T & T/T	Asymptomatic smokers: FEV_1 % predicted 109.4 ± 16.5 L (30)	22.1		
GSTT1 NULL Homozygous deletion resulting in complete lack of activity						
Calikoglu 2006	Turkish men	Null	Stable COPD patients (149)	14.6	1.22 (0.65, 2.29)	+0++
			Healthy controls (150)	17.3	*ref*	
Chan-Yeung 2007	Chinese	Null	COPD patients (163)	54.6	0.83 (0.54, 1.29)	+0++
			Healthy controls (163)	59.0	*ref*	
Cheng	Taiwanese	Null	COPD patients (184)	53.8	0.9 (0.6, 1.6)	+0++

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
2004			Asymptomatic ever smokers (212)	52.8	*ref*	
Yim 2000	Korean	Null	COPD patients (includes never-smokers) (83)	55.0	0.77 (0.41, 1.45)	-0+-
			Asymptomatic smokers (76)	62.0	*ref*	
Budhi 2003	Japanese	Null	Heavy smokers with emphysematous changes (63)	42.9	1.06 (0.6, 1.9)	00++
			Heavy smokers without emphysematous changes (172)	41.3	*ref*	
He 2002	Predominantly White North American	Null	Smokers with steepest decline in FEV_1 (299)	30.4	1.23 (0.83, 1.82)	++++
			Smokers with slowest decline in FEV_1 (322)	25.1	*ref*	
He 2004	White North American	Null	Smokers with lowest FEV_1 % predicted (544)	22.3	1.3 (0.94, 1.82)	++++
			Smokers with highest FEV_1 % predicted (544)	17.8	*ref*	
Tkacova 2004	Slovakian lung cancer patients	Null	GSTT1 Null: Mean FEV_1 % predicted = 81.4 ± 4.9 (110)	21.8	p>0.05	-+++
			GSTT1 Non-null: Mean FEV_1 % predicted = 79.3 ± 2.3 (110)	78.2		
Tkacova 2004	Slovakian lung cancer patients	Null	GSTT1 Null: Mean FVC % predicted = 89.3 ± 3.3 (110)	21.8	p>0.05	-+++
			GSTT1 Non-null: Mean FVC % predicted = 85.6 ± 1.7 (110)	78.2		
Imboden 2007	European Caucasians (predominantly)	Null	Linear regression of genotype on FEV_1 ml decline/yr (4686)	18.0	Men:-5.3(-8.4,-2.1)[viii] Women:-0.3(-2.5,1.8)	++++
Imboden 2007	European Caucasians (predominantly)	Null	Linear regression of genotype on FVC ml decline/yr (4686)	18.0	Men:-5.2(-7.4,-1.3)[viii] Women:-1.6(-4.5,1.3)	++++
Imboden 2007	European Caucasians (predominantly)	Null	Linear regression of genotype on FEF_{25-75} ml decline/yr (4686)	18.0	Men:-5.0(-11.7, 1.8)[viii] Women:3.4 (-1.5,8.3)	++++

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
mGST1 G→A in the 3' UTR. *rs11875*						
Hersh 2006	White American emphysema patients		Linear regression: additive genetic model and FEV_1 % predicted (304)	NA	p> 0.10	+0++
mGST1 G→A in the 3' UTR. *rs2160512*						
Hersh 2006	White American emphysema patients		Linear regression: additive genetic model and FEV_1 % predicted (304)	NA	p> 0.10	+0++
mGST1 A→G in intron 1. *rs2239675*						
Hersh 2006	White American emphysema patients		Linear regression: additive genetic model and FEV_1 % predicted (304)	NA	p> 0.10	+0++
mGST1 A→T in intron 3. *rs2287152*						
Hersh 2006	White American emphysema patients		Linear regression: additive genetic model and FEV_1 % predicted (304)	NA	p> 0.10	+0++
Thioredoxin Metabolism						
(No studies)						
Other Antioxidant Enzymes						
SOD1 A→C substitution in intron 3 (+35), adjacent to a splice site [51] *rs2234694*						
Young 2006	European descent	A/A	COPD patients (230)	92.0	*Ref*	++++
			Asymptomatic smokers (210)	90.0		
		A/C	COPD patients (230)	7.0	0.75 (0.38, 1.50)	
			Asymptomatic smokers (210)	9.0		
		C/C	COPD patients (230)	1.0	*0.89 (0.12, 6.39)*[ix]	

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
			Asymptomatic smokers (210)	1.0		
SOD2 Val16Ala T→C at nucleotide 47, changes Val to Ala at 16, possibly less efficient localisation to mitochondria [52] *rs4880*						
Young 2006	European descent	T/T	COPD patients (230)	23.0	*Ref*	++++
			Asymptomatic smokers (210)	22.0		
		T/C	COPD patients (230)	52.0	0.92 (0.58, 1.48)	
			Asymptomatic smokers (210)	54.0		
		C/C	COPD patients (230)	25.0	0.97 (0.56, 1.68)	
			Asymptomatic smokers (210)	24.0		
Mak 2007	Chinese	T/T	COPD patients (165)	72.6	*Ref*	++++
			Asymptomatic ever smokers (165)	79.9		
		T/C & C/C	COPD patients (165)	27.4	1.50 (0.90, 2.51)	
			Asymptomatic ever smokers (165)	20.1		
SOD3 Arg213Gly C→G substitution at nucleotide 760, changes Arg to Gly at 213, resulting in decreased heparin affinity and increased plasma SOD3 content [53] *rs1799895*						
Juul 2006	Danish	C/C	COPD patients (978)	99.0	*Ref*	++++
			Asymptomatic smokers and nonsmokers (7604)	97.4		
		C/G	COPD patients (978)	1.5	0.58 (0.34, 0.99)	
			Asymptomatic smokers and nonsmokers (7604)	2.6		
		G/G	COPD patients (978)	0.0	NA	
			Asymptomatic smokers and nonsmokers (7604)	3.0		
Young 2006	European descent	C/C	COPD patients (230)	98.0	*ref*	++++
			Asymptomatic smokers (210)	91.0		
			Healthy blood donors (190)	96.0		

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
		C/G	COPD patients (230)	2.0	0.25 (0.09, 0.69) [vii] 0.67 (0.20, 2.24) [v]	
			Asymptomatic smokers (210)	8.0		
			Healthy blood donors (190)	3.0		
		G/G	COPD patients (230)	0.0	NA	
			Asymptomatic smokers (210)	1.0		
			Healthy blood donors (190)	1.0		
Juul 2006	Danish	C/C	Incidence of COPD hospitalization or death: 17%		*ref*	++++
		C/G	Incidence of COPD hospitalization or death: 7%		0.3 (0.1, 0.8)	
		G/G	Incidence of COPD hospitalization or death: 0%		NA	
Juul 2006	Danish	C/C	Mean FEV_1 % predicted: 85.0 ± 18% (8879)	97.6	p= 0.41	++++
		C/G	Mean FEV_1 % predicted: 85.5 ± 18% (8879)	2.4		
Juul 2006	Danish	C/C	Mean FEV_1 % predicted (Smokers): 84.0 ± 19% (6906)	97.5	p=0.17	++++
		C/G	Mean FEV_1 % predicted (Smokers): 86.7 ± 20% (6906)	2.5		
Juul 2006	Danish	C/C	Mean FVC % predicted: 87.3 ± 16% (8879)	97.6	p=0.32	++++
		C/G	Mean FVC % predicted: 90.5 ± 16% (8879)	2.4		
Juul 2006	Danish	C/C	Mean FVC % predicted (Smokers): 88.5 ± 16% (6906)	97.5	p=0.84	++++
		C/G	Mean FVC % predicted (Smokers): 89.4 ± 16% (6906)	2.5		
Juul 2006	Danish	C/C	Mean FEV_1 /FVC: 77.1 ± 9 (8879)	97.6	p=0.11	++++
		C/G	Mean FEV_1 /FVC: 78.2 ± 9 (8879)	2.4		
Juul 2006	Danish	C/C	Mean FEV_1 /FVC (Smokers): 75.7 ± 11 (6906)	97.5	p=0.04	++++
		C/G	Mean FEV_1 /FVC (Smokers): 77.8 ± 9 (6906)	2.5		
CAT A→T in the promoter region (position -21), associated with acatalesemia [54] *rs7943316*						
Young	European descent	A/A	COPD patients (230)	52.0	*ref*	++++

TABLE A.3 (continued)

Reference	Population description[i]	Genotype	Subgroups (n)[ii]	Genotype Frequency (%)	Odds Ratio (95% CI)	Quality Score (PEOC)[iii]
2006			Asymptomatic smokers (210)	48.0		
		A/T	COPD patients (230)	41.0	0.90 (0.61, 1.33)	
			Asymptomatic smokers (210)	42.0		
		T/T	COPD patients (230)	7.0	0.64 (0.32, 1.29)	
			Asymptomatic smokers (210)	10.0		
CAT C→T in the promoter region (position -262), associated with increased expression in red blood cells [55] *rs1001179*						
Mak 2007	Chinese	C/C	COPD patients (all ever smokers) (165)	90.9	*ref*	++++
			Asymptomatic ever smokers (165)	93.3		
		C/T & T/T	COPD patients (all ever smokers) (165)	9.1	1.40 (0.63, 3.14)	
			Asymptomatic ever smokers (165)	6.7		

i Unless mentioned, all study populations included both men and women.
ii Reference group is the last subgroup listed.
iii Scores (+,0,-) are given for each of the following categories in this order: Population Selection, Exposure, Outcome Determination, and Covariate Handling.
iv Reference group is participants with lung cancer only
v Reference group is healthy blood donors
vi Allele frequencies given instead of genotype frequencies
vii Reference group is asymptomatic smokers
viii Beta coefficient (95% CI) given for regression models
ix Estimate based on group(s) with fewer than 5 participants

TABLE A.4

GENE EXPRESSION STUDIES OF GENES RELATED TO ANTIOXIDANT FUNCTION IN PATIENTS WITH COPD IN COMPARISON TO CONTROLS, AND IN SMOKERS COMPARED TO NONSMOKERS.

GENETIC VARIATION AND GENE EXPRESSION IN ANTIOXIDANT-RELATED ENZYMES AND RISK OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A SYSTEMATIC REVIEW

Expression studies used a variety of assay types, limiting direct comparability across studies. To facilitate comparisons, the ratio of expression in one group to expression in the reference group was given. If not provided in the publication, ratios were calculated (using numeric estimates derived from graphs, where necessary). Quality of studies was evaluated in four categories of study features: population selection, exposure assessment, outcome assessment, and handling of covariates. Each study was scored on whether the presented methods did ("+") or did not ("-") appropriately address potential bias in that category ("+"), or whether risk of bias could not be determined from given information ("0"). Results of the expression studies are also sorted within groups of genes and then by tissue type, as follows: from small airways (alveolar epithelium), to large airways (central bronchus epithelium), to non-epithelial tissues.

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
Glutathione Synthesis							
GGT							
(No studies)							
GCLC							
Rahman 2000	Alveolar epithelium [v]	In situ hybridisation	COPD patients (11) Asymptomatic ever smokers (12)	1.81 ± 0.09 [GR] 1.42 ± 0.14	1.27 *ref*	p<0.04	-++-
Lin 2005	Alveoli [v]	In situ hybridisation	COPD patients (12) Patients without COPD (10)	0.25 ± 0.05 [GR] 0.14 ± 0.03	1.79 *ref*	p<0.05	-+0-
Lin 2005	Alveoli [v]	Immunohistochemistry	COPD patients (12) Patients without COPD (10)	0.20 ± 0.04 [GR] 0.12 ± 0.04	1.67 *ref*	p<0.05	-+0-
Harju 2002	Bronchioli epithelium [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	1.5 ± 0.2 [GR] 1.4 ± 0.1 1.4 ± 0.2	1.07 1.00 *ref*	NS NS	-++-
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		2.29 *ref*	p=0.0004	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	1100 [vi] 1199 926 960 728	1.51 1.65 1.27 1.32 *ref*		-++-
Rahman 2000	Airway epithelial cells [v]	In situ hybridisation	COPD patients (11) Asymptomatic ever smokers (12)	1.82 ± 0.17 [GR] 1.42 ± 0.12	1.28 *ref*	p=0.075	-++-
Lin 2005	Bronchi [v]	In situ hybridisation	COPD patients (12) Patients without COPD (10)	0.31 ± 0.05 [GR] 0.17 ± 0.04	1.82 *ref*	p<0.05	-+0-
Lin 2005	Bronchi [v]	Immunohistochemistry	COPD patients (12) Patients without COPD (10)	0.18 ± 0.02 [GR] 0.10 ± 0.03	1.80 *ref*	p<0.05	-+0-
Harju 2002	Central bronchus epithelium [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20)	1.6 ± 0.2 [GR] 1.8 ± 0.2	0.76 0.86	p=0.015 NS	-++-

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
			Asymptomatic nonsmokers (13)	2.1 ± 0.2	*ref*		
Neurohr 2003	Alveolar macrophages (bronchoalveolar lavage)	RT-PCR	Asymptomatic smokers (8) Asymptomatic nonsmokers (8)	0.6 ± 0.1 RCE 0.9 ± 0.2	0.67 *ref*	NS	00+-
Harju 2002	Alveolar macrophages [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	0.8 ± 0.1 GR 0.4 ± 0.1 1.5 ± 0.2	0.52 0.28 *ref*	p=0.053 p<0.001	-++-
Lin 2005	Inflammatory cells [v]	In situ hybridisation	COPD patients (12) Patients without COPD (10)	0.28 ± 0.06 GR 0.20 ± 0.05	1.40 *ref*	p<0.05	-+0-
Lin 2005	Inflammatory cells [v]	Immunohistochemistry	COPD patients (12) Patients without COPD (10)	0.25 ± 0.06 GR 0.14 ± 0.04	1.79 *ref*	p<0.05	-+0-
GCLM							
Harju 2002	Bronchioli epithelium [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	0.8 ± 0.05 GR 0.9 ± 0.1 1.1 ± 0.1	0.73 0.82 *ref*	NS NS	-++-
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		2.5 1.0	p=0.04	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	788 86 [vi] 974 90 712 86 628 83 429 66	1.84 1.30 2.27 1.36 1.66 1.30 1.46 1.26 *ref* *ref*		-++-
Harju 2002	Central bronchus epithelium [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	1.0 ± 0.1 GR 1.0 ± 0.1 1.6 ± 0.2	0.63 0.63 *ref*	p=0.006 p=0.021	-++-
Harju 2002	Alveolar macrophages [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	1.1 ± 0.2 GR 0.6 ± 0.1 1.6 ± 0.2	0.69 0.38 *ref*	NS p<0.001	-++-

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
Neurohr 2003	Alveolar macrophages (bronchoalveolar lavage)	RT-PCR	Asymptomatic smokers (8) Asymptomatic nonsmokers (8)	0.6 ± 0.1 [RCE] 2.8 ± 0.4	0.21 *ref*	p<0.01	00+-
GSS							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.27 *ref*	p=0.08	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	369 [vi] 398 388 355 373	0.99 1.07 1.04 0.95 *ref*		-++-
Glutathione Antioxidant Activity and Recycling							
GPX							
GPX1							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.15 *ref*	p=0.61	++++
GPX2							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		5.09 *ref*	p<0.00001	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	1551 [vi] 1784 1715 1437 427	3.63 4.18 4.02 3.37 *ref*		-++-
GPX3							
Comhair 2000	Airway epithelial cells (bronchoscopy)	Northern blot	Asymptomatic smokers (11) Asymptomatic nonsmokers (12)	18 ± 3 [RCE] 8.0 ± 0.8	2.25 *ref*	p<0.05	+0+0
Hackett	Airway epithelial	Microarray	Asymptomatic smokers (13)		1.86	p=0.007	++++

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
2003	cells (bronchoscopy)		Asymptomatic nonsmokers (9)		*ref*		
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	568 [vi] 665 536 526 454	1.25 1.46 1.18 1.16 *ref*		-++-
Tomaki 2007	Peripheral lung tissue (lung resection) [v]	RT-PCR	COPD patients (smokers) (14) Smokers (9) Nonsmokers (10)	0.831 ± 0.206 1.030 ± 0.117 0.736 ± 0.178	1.13 1.40 *ref*	NS NS	-++-
Comhair 2000	Alveolar Macrophages (bronchoalveolar lavage)	Northern blot	Asymptomatic smokers (11) Asymptomatic nonsmokers (12)	44 ± 10 [RCE] 24 ± 3	1.83 *ref*	p<0.05	+0+0
GPX4							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.10 *ref*	p=0.61	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	1571 [vi] 2102 1463 1526 1650	0.95 1.27 0.89 0.92 *ref*		-++-
GPX5							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	108 [vi] 99 110 117 97	1.11 1.02 1.13 1.21 *ref*		-++-
GPX7							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7)	82 [vi] 84	0.94 0.97		-++-

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
			COPD patients, stage 2-4 (13)	85	0.98		
			Asymptomatic smokers (18)	84	0.96		
			Nonsmokers (18)	87	*ref*		
GLRX							
GLRX							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	364 [vi]	1.19		-++-
			COPD patients, stage 1 (7)	291	0.95		
			COPD patients, stage 2-4 (13)	337	1.10		
			Asymptomatic smokers (18)	302	0.99		
			Nonsmokers (18)	306	*ref*		
Peltoniemi 2006	Lung tissue homogenate [v] (resection/transplantation)	Western Blot Analysis	COPD patients, stage I-II (9)		0.86	NS/p=0.045[vii]	-0+-
			COPD patients, stage IV (6)		0.81	NS/p=0.022 [vii]	
			Asymptomatic smokers (9)		1.09	NS	
			Nonsmokers (8)		*ref*		
Peltoniemi 2006	Sputum supernatants	Western Blot Analysis	COPD patients (exacerbation) (7)		1.33	P=0.013	-0+-
			Stable COPD, stage 0-I (17)		1.16	NS	
			Asymptomatic smokers (11)		1.07	NS	
			Nonsmokers (15)				
GSR							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13)		2.31	p=0.03	++++
			Asymptomatic nonsmokers (9)		*ref*		
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	340 [vi]	1.62		-++-
			COPD patients, stage 1 (7)	372	1.77		
			COPD patients, stage 2-4 (13)	368	1.75		
			Asymptomatic smokers (18)	304	1.45		
			Nonsmokers (18)	210	*ref*		
G6PD							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13)		1.85	p<0.00001	++++
			Asymptomatic nonsmokers (9)		*ref*		

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	291 [vi] 345 286 269 155	1.88 2.23 1.85 1.74 *ref*		-++-
Heguy 2006	Alveolar Macrophages (bronchoscopy)	Microarray	Asymptomatic smokers (5) Asymptomatic nonsmokers (5)		2.1 1.0	p<0.011	++++
Glutathione Conjugation and Transport							
GST							
GSTA1							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	4881 [vi] 5070 3881 4722 4274	1.14 1.19 0.91 1.10 *ref*		-++-
Tomaki 2007	Peripheral lung tissue (lung resection) [v]	RT-PCR	COPD patients (smokers) (14) Smokers (9) Nonsmokers (10)	0.126 ± 0.019 0.229 ± 0.044 0.136 ± 0.033	0.93 1.68 *ref*	NS p<0.05	-++-
GSTA2							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.48 *ref*	p=0.0025	++++
GSTM3							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.44 *ref*	p=0.38	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	135 [vi] 205 174 127 86	1.57 2.38 2.02 1.48 *ref*		-++-

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]		T Test	Quality Score (PEOC)[iv]
GSTM4								
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.5f4 *ref*		p=0.21	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	440 460 523 454 549	199 [vi] 209 222 208 221	0.80 0.84 0.95 0.83 *ref*	0.90 0.95 1.00 0.94 *ref*	-++-
GSTO1								
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	789 [vi] 791 647 653 614	1.29 1.29 1.05 1.06 *ref*			-++-
Harju 2007	Alveolar epithelium (resection [v] or lung transplantation)	Immunohistochemistry	COPD, stage I-II (CS) (26) COPD, stage IV (ex-smokers) (8) Asymptomatic CS (22) Lifelong non-smokers (16)	0.6 ± 0.1^{GR} 1.0 ± 0.1 0.5 ± 0.1 0.5 ± 0.1	1.2 2.0 1.0 *ref*		NS p<0.05 NS	-+00
Harju 2007	Bronchial epithelium (resection [v] or lung transplantation)	Immunohistochemistry	COPD, stage I-II (CS) (26) COPD, stage IV (ex-smokers) (8) Asymptomatic CS (22) Lifelong non-smokers (16)	0.2 ± 0.1^{GR} 0.6 ± 0.1 0.3 ± 0.1 0.3 ± 0.1	0.7 2.0 1.0 *ref*		NS NS NS	-+00
Harju 2007	Lung tissue homogenate (resection) [v]	Western Blot Analysis	COPD patients (CS) (17) Asymptomatic CS (5) Lifelong non-smokers (9)		0.80 1.01 *ref*		p=0.003 NS	-+00
Harju 2007	Induced sputum [v]	Western Blot Analysis	COPD patients (CS) (15) Asymptomatic CS (5) Lifelong non-smokers (6)		0.67 0.97 *ref*		p=0.023 NS	-+00
Harju 2007	Alveolar macrophages (resection [v] or lung transplantation)	Immunohistochemistry	COPD, stage I-II (CS) (26) COPD, stage IV (ex-smokers) (8) Asymptomatic CS (22) Lifelong non-smokers (16)	1.8 ± 0.1^{GR} 1.7 ± 0.1 1.4 ± 0.1 1.5 ± 0.1	1.2 1.1 0.9 *ref*		NS NS NS	-+00

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
Harju 2007	Pulmonary blood vessels (resection [v] or lung transplantation)	Immunohistochemistry	COPD, stage I-II (CS) (26) COPD, stage IV (ex-smokers) (8) Asymptomatic CS (22) Lifelong non-smokers (16)	0.15 ± 0.2[GR] 0.5 ± 0.2 0.15 ± 0.0 0.3 ± 0.1	0.5 1.7 0.5 *ref*	NS NS NS	-+00
GSTP1							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		0.97 *ref*	p=0.98	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	2983 [vi] 3448 2711 2895 2837	1.05 1.22 0.96 1.02 *ref*		-++-
GSTT1							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.60 *ref*	p=0.25	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	214 [vi] 237 210 221 233	0.92 1.02 0.90 0.95 *ref*		-++-
GSTT2							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.06 *ref*	p=0.89	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	77 [vi] 63 67 72 71	1.08 0.89 0.94 1.01 *ref*		-++-
GSTZ1							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.01 *ref*	p=0.92	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7)	179 [vi] 187	1.04 1.09		-++-

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
			COPD patients, stage 2-4 (13)	186	1.08		
			Asymptomatic smokers (18)	179	1.04		
			Nonsmokers (18)	172	*ref*		
mGST1							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13)		1.48	p=0.16	++++
			Asymptomatic nonsmokers (9)		*ref*		
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	1411 [vi]	1.56		-++-
			COPD patients, stage 1 (7)	1417	1.56		
			COPD patients, stage 2-4 (13)	1252	1.38		
			Asymptomatic smokers (18)	1039	1.15		
			Nonsmokers (18)	907	*ref*		
mGST2							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13)		1.03	p=0.92	++++
			Asymptomatic nonsmokers (9)		*ref*		
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	434 [vi]	0.95		-++-
			COPD patients, stage 1 (7)	524	1.14		
			COPD patients, stage 2-4 (13)	425	0.93		
			Asymptomatic smokers (18)	382	0.83		
			Nonsmokers (18)	458	*ref*		
Thioredoxin Metabolism							
TXN							
TXN1							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13)		1.39	p=0.07	++++
			Asymptomatic nonsmokers (9)		*ref*		
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	2259 [vi]	2.11		-++-
			COPD patients, stage 1 (7)	2586	2.42		
			COPD patients, stage 2-4 (13)	1966	1.84		
			Asymptomatic smokers (18)	1474	1.38		
			Nonsmokers (18)	1069	*ref*		
TXN2							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	274 [vi]	0.95		-++-
			COPD patients, stage 1 (7)	296	1.02		
			COPD patients, stage 2-4 (13)	278	0.96		

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
			Asymptomatic smokers (18)	260	0.90		
			Nonsmokers (18)	289	*ref*		
TXNRD							
TXNRD1							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13)		2.20	p=0.0007	++++
			Asymptomatic nonsmokers (9)		*ref*		
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	1650 [vi]	1.93		-++-
			COPD patients, stage 1 (7)	1879	2.20		
			COPD patients, stage 2-4 (13)	1564	1.83		
			Asymptomatic smokers (18)	1443	1.69		
			Nonsmokers (18)	853	*ref*		
TXNRD2							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	114 [vi]	1.00		-++-
			COPD patients, stage 1 (7)	116	1.02		
			COPD patients, stage 2-4 (13)	117	1.03		
			Asymptomatic smokers (18)	111	0.97		
			Nonsmokers (18)	114	*ref*		
TXNRD3							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	133 [vi]	1.02		-++-
			COPD patients, stage 1 (7)	135	1.04		
			COPD patients, stage 2-4 (13)	132	1.02		
			Asymptomatic smokers (18)	131	1.01		
			Nonsmokers (18)	130	*ref*		
PRDX							
PRDX1							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	5786 [vi]	1.50		-++-
			COPD patients, stage 1 (7)	6883	1.79		
			COPD patients, stage 2-4 (13)	5558	1.45		
			Asymptomatic smokers (18)	4923	1.28		
			Nonsmokers (18)	3845	*ref*		
PRDX2							
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	329	452 [vi]	1.13	0.95	-++-
			COPD patients, stage 1 (7)	375	483	1.28	1.02	

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]		T Test	Quality Score (PEOC)[iv]
			COPD patients, stage 2-4 (13)	313	446	1.07	0.94	
			Asymptomatic smokers (18)	287	467	0.98	0.99	
			Nonsmokers (18)	292	474	*ref*	*ref*	
PRDX3								
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	703 [vi]	0.90			-++-
			COPD patients, stage 1 (7)	1067	1.36			
			COPD patients, stage 2-4 (13)	625	0.80			
			Asymptomatic smokers (18)	584	0.74			
			Nonsmokers (18)	784	*ref*			
PRDX4								
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13)		1.16		p=0.55	++++
			Asymptomatic nonsmokers (9)		*ref*			
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	551 [vi]	1.22			-++-
			COPD patients, stage 1 (7)	670	1.48			
			COPD patients, stage 2-4 (13)	517	1.14			
			Asymptomatic smokers (18)	448	0.99			
			Nonsmokers (18)	452	*ref*			
PRDX5								
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	1798 [vi]	0.71			-++-
			COPD patients, stage 1 (7)	1751	0.69			
			COPD patients, stage 2-4 (13)	1940	0.76			
			Asymptomatic smokers (18)	1940	0.76			
			Nonsmokers (18)	2543	*ref*			
PRDX6								
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18)	1029 [vi]	0.93			-++-
			COPD patients, stage 1 (7)	1243	1.12			
			COPD patients, stage 2-4 (13)	998	0.90			
			Asymptomatic smokers (18)	1024	0.92			
			Nonsmokers (18)	1111	*ref*			
Other Antioxidant Enzymes								

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
SOD							
SOD							
Yigla 2007	Bronchoalveolar lavage fluid	Enzyme Assay	COPD patients (smokers) (10) COPD patients (nonsmokers) (10) Asymptomatic smokers (10) Nonsmokers without COPD (10)	1.25 ± 0.31 2.00 ± 0.80 0.98 ± 0.12 1.07 ± 0.16	1.17 1.87 0.92 *ref*	NS NS NS	-+00
SOD1							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.26 *ref*	p=0.28	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	2446 [vi] 2946 1996 2030 2277	1.07 1.29 0.88 0.89 *ref*		-++-
Harju 2004	Lung tissue homogenate [v]	Western blot	Smokers (with moderate to no COPD) (4) Lifelong nonsmokers (3)		1.25 *ref*	NS	-+0-
Tomaki 2007	Peripheral lung tissue (lung resection) [v]	RT-PCR	COPD patients (smokers) (14) Smokers (9) Nonsmokers (10)	0.045 ± 0.007 0.047 ± 0.010 0.058 ± 0.008	0.78 0.81 *ref*	NS NS	-++-
Kondo 1994	Alveolar Macrophages (bronchoalveolar lavage)	Northern blot	Asymptomatic elderly male smokers (3) Asymptomatic elderly male nonsmokers (3)	No difference[viii]	1.0 *ref*		++00
SOD2							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		0.8 *ref*	p=0.3	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18)	153 84 130 85	138 [vi] 104 114 98	1.38 0.76 1.17 0.77	0.91 0.68 0.75 0.64	-++-

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]	T Test	Quality Score (PEOC)[iv]
			Nonsmokers (18)	111	152 *ref*	*ref*	
Harju 2004	Central bronchus epithelium [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	1.8 ± 0.2 [GR] 1.4 ± 0.2 0.9 ± 0.2	2.0 1.6 *ref*	p=0.041 NS	-+0-
Harju 2004	Bronchioli epithelium [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	0.5 ± 0.2 [GR] 0.3 ± 0.1 0.4 ± 0.2	1.25 0.75 *ref*	NS NS	-+0-
Harju 2004	Alveolar epithelium [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	0.6 ± 0.2 [GR] 0.5 ± 0.1 0.05 ± 0.08	12.0 10.0 *ref*	p=0.01 p=0.022	-+0-
Harju 2004	Lung tissue homogenate [v]	Western blot	Smokers (moderate/no COPD) (4) Lifelong nonsmokers (3)		1.33[vi] *ref*		-+0-
Tomaki 2007	Peripheral lung tissue (lung resection) [v]	RT-PCR	COPD patients (smokers) (14) Smokers (9) Nonsmokers (10)	0.660 ± 0.139 0.517 ± 0.122 0.384 ± 0.090	1.72 1.35 *ref*	NS NS	-++-
Harju 2004	Alveolar macrophages [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	1.8 ± 0.2 [GR] 1.7 ± 0.1 1.1 ± 0.3	1.6 1.5 *ref*	NS NS	-+0-
Harju 2004	Pulmonary blood vessels [v]	Immunohistochemistry	COPD patients (smokers) (22) Asymptomatic smokers (20) Asymptomatic nonsmokers (13)	0.03 ± 0.04 [GR] 0.04 ± 0.03 0.07 ± 0.07	0.4 0.6 *ref*	NS NS	-+0-
SOD3							
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.17 *ref*	p=0.52	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	174 [vi] 158 171 169 161	1.08 0.98 1.06 1.05 *ref*		-++-
Harju 2004	Lung tissue homogenate [v]	Western blot	Smokers (moderate/no COPD) (4) Lifelong nonsmokers (3)		1.21[vi] *ref*		-+0-

TABLE A.4 (continued)

Reference	Tissue [i] (Collection method)	Assay	Subgroups [ii] (n)	Mean ± SEM	Ratio [iii]		T Test	Quality Score (PEOC)[iv]
Tomaki 2007	Peripheral lung tissue (lung resection) [v]	RT-PCR	COPD patients (smokers) (14) Smokers (9) Nonsmokers (10)	0.187 ± 0.013 0.234 ± 0.013 0.221 ± 0.018	0.85 1.06 *ref*		NS NS	-++-
CAT								
Hackett 2003	Airway epithelial cells (bronchoscopy)	Microarray	Asymptomatic smokers (13) Asymptomatic nonsmokers (9)		1.36 *ref*		p=0.27	++++
Pierrou 2007	Bronchial epithelial cells (bronchoscopy)	Microarray	COPD patients, stage 0 (18) COPD patients, stage 1 (7) COPD patients, stage 2-4 (13) Asymptomatic smokers (18) Nonsmokers (18)	399 487 439 388 504	1331 [vi] 1992 1409 1412 1463	0.67 0.97 0.87 0.77 *ref*	0.91 1.36 0.96 0.97 *ref*	-++-
Tomaki 2007	Peripheral lung tissue (lung resection) [v]	RT-PCR	COPD patients (smokers) (14) Smokers (9) Nonsmokers (10)	0.165 ± 0.013 0.233 ± 0.026 0.251 ± 0.025	0.66 0.93 *ref*		p<0.01 NS	-++-

[i] Lung tissue sample unless otherwise indicated
[ii] Reference group for all comparisons is the last subgroup listed: the ratios and significance tests listed are for the subgroup compared to the reference group.
[iii] Ratio of subgroup to reference group (last group listed)
[iv] Quality scores (+,0,-) given for the following categories in this order: Population Selection, Exposure Status, Outcome Determination, and Covariate Handling.
[v] All study participants undergoing lung resection for tumor (non-tumor tissue tested)
[vi] Median values reported, so t-test could not be performed. When multiple probe sets gave similar patterns of regulation, the average is listed. When multiple probe sets gave different patterns, they are listed separately in divided column.
[vii] Comparison group for this result is asymptomatic smokers
[viii] No quantitative data given for this result
[GR] Semi-quantitative grading of stain intensity
[RCE] Ratio with a control enzyme

SUPPLEMENT A.5

SUPPLEMENTAL BIBLIOGRAPHY: REFERENCES FOR TABLES A.2-4

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TABLE A.6

DESCRIPTION OF CANDIDATE GENES
GENETIC VARIATION IN ANTIOXIDANT ENZYMES AND LUNG
FUNCTION IN THE HEALTH, AGING, AND BODY COMPOSITION STUDY

Gene Name (Abbreviation)	Functional Group	Gene Length (kb)	LDU across Gene Af Amer	LDU across Gene Eur Amer	SNPs	NS SNPs
Catalase (CAT)	Catalase	33	1.16	0.06	7	0
Gamma-glutamyl Transferase 1 (GGT1)	GSH Synthesis	45	2.05	1.83	3	0
Glucose-6-Phosphate Dehydrogenase (G6PD)	Reducing Equivalents	16	0	0	1	0
Glutamate-cysteine ligase (catalytic) (GCLC)	GSH Synthesis	48	3.85	1.71	15	0
Glutamate-cysteine ligase (modulatory) (GCLM)	GSH Synthesis	22	0.16	0.18	6	0
Glutaredoxin (GLRX)	Disulfide Reductase	9	0.74	0.21	5	0
Glutaredoxin 2 (GLRX2)	Disulfide Reductase	10	0	0	6	0
Glutathione Peroxidase 1 (GPX1)	Peroxidase Activity	1.2	0.02	0	3	0
Glutathione Peroxidase 2 (GPX2)	Peroxidase Activity	3.7	0.12	0	5	0
Glutathione Peroxidase 3 (GPX3)	Peroxidase Activity	8.5	1.11	0.45	4	0
Glutathione Peroxidase 4 (GPX4)	Peroxidase Activity	2.9	0.78	0.14	5	0
Glutathione Peroxidase 7 (GPX7)	Peroxidase Activity	6.7	0	0	5	0
Glutathione Reductase (GSR)	Disulfide Reductase	49	1.96	0.10	9	1
Glutathione S-Transferase A1 (GSTA1)	Glutathione S-Transferase	12	0.01	0	3	0
Glutathione S-Transferase A2 (GSTA2)	Glutathione S-Transferase	13	1.19	0.19	3	1
Glutathione S-Transferase A3 (GSTA3)	Glutathione S-Transferase	13	0	0.03	6	1
Glutathione S-Transferase A4 (GSTA4)	Glutathione S-Transferase	17	0	0.48	6	0
Glutathione S-Transferase A5 (GSTA5)	Glutathione S-Transferase	14	0.18	0	7	1
Glutathione S-Transferase K1 (GSTK1)	Glutathione S-Transferase	5.6	0.15	0.01	1	0
Glutathione S-Transferase M2 (GSTM2)	Glutathione S-Transferase	7.2	0	0.55	3	0
Glutathione S-Transferase M3 (GSTM3)	Glutathione S-Transferase	6.5	0.283	0.089	6	1
Glutathione S-Transferase M4 (GSTM4)	Glutathione S-Transferase	9.4	0.235	0.664	4	0
Glutathione S-Transferase O1 (GSTO1)	Glutathione S-Transferase	13	0	0.005	4	0
Glutathione S-Transferase O2 (GSTO2)	Glutathione S-Transferase	31	0.863	0.082	9	1

TABLE A.6 (continued)

Gene Name (Abbreviation)	Functional Group	Gene Length (kb)	LDU across Gene Af Amer	LDU across Gene Eur Amer	SNPs	NS SNPs
Glutathione S-Transferase P1 (GSTP1)	Glutathione S-Transferase	2.8	0.055	0.083	8	2
Glutathione S-Transferase Z1 (GSTZ1)	Glutathione S-Transferase	8.1	0	0	6	2
Glutathione Synthetase (GSS)	GSH Synthesis	11	0.199	0.147	7	0
Heme-Oxygenase 1 (HMOX1)	Heme-Oxygenase	13	0.234	0.206	5	0
Heme-Oxygenase 2 (HMOX2)	Heme-Oxygenase	34	0	0.099	6	0
Isocitrate Dehydrogenase 1 (IDH1)	Reducing Equivalents	19	1.188	0.443	7	1
Isocitrate Dehydrogenase 2 (IDH2)	Reducing Equivalents	19	0.849	2.134	7	0
Isocitrate Dehydrogenase 3A (IDH3A)	Reducing Equivalents	21	0	0.192	5	0
Isocitrate Dehydrogenase 3B (IDH3B)	Reducing Equivalents	5.8	0.571	0.116	4	0
Isocitrate Dehydrogenase 3G (IDH3G)	Reducing Equivalents	8.7	0	0.236	5	0
Microsomal Glutathione S-Transferase 1 (mGST1)	Glutathione S-Transferase	17	1.151	2.013	8	0
Microsomal Glutathione S-Transferase 2 (mGST2)	Glutathione S-Transferase	39	8.838	1.303	11	0
Microsomal Glutathione S-Transferase 3 (mGST3)	Glutathione S-Transferase	24	3.25	1.293	11	0
Peroxiredoxin 1 (PRDX1)	Peroxidase Activity	11	0	0	3	0
Peroxiredoxin 2 (PRDX2)	Peroxidase Activity	5	0.005	0	6	0
Peroxiredoxin 3 (PRDX3)	Peroxidase Activity	11	0.271	0.107	6	0
Peroxiredoxin 4 (PRDX4)	Peroxidase Activity	19	0.218	0.039	6	0
Peroxiredoxin 5 (PRDX5)	Peroxidase Activity	3.7	0.145	0	6	1
Peroxiredoxin 6 (PRDX6)	Peroxidase Activity	11	0.005	0	6	0
Selenoprotein P 1 (SEPP1)	Selenoprotein	12	0.154	0.312	6	1
Selenoprotein W 1 (SEPW1)	Selenoprotein	6.1	0.032	0.131	7	0
Superoxide Dismutase 1 (SOD1)	Superoxide Dismutase	9.3	0	0	6	0
Superoxide Dismutase 2 (SOD2)	Superoxide Dismutase	14	0.074	0.143	6	1
Superoxide Dismutase 3 (SOD3)	Superoxide Dismutase	6.4	2.603	2.277	10	1
Thioredoxin 1 (TXN)	Thioredoxin	12	1.524	1.078	5	0
Thioredoxin 2 (TXN2)	Thioredoxin	15	1.031	0.308	5	0

TABLE A.6 (continued)

Gene Name (Abbreviation)	Functional Group	Gene Length (kb)	LDU across Gene Af Amer	LDU across Gene Eur Amer	SNPs	NS SNPs
Thioredoxin Reductase 1 (TXNRD1)	Disulfide Reductase	63	0	0	8	0
Thioredoxin Reductase 2 (TXNRD2)	Disulfide Reductase	66	3.599	3.071	12	1
Thioredoxin Reductase 3 (TXNRD3)	Disulfide Reductase	52	0.892	0.356	4	0

*Abbreviations: African American (Af Amer), European American (Eur Amer), Linkage Disequilibrium Units (LDU), Nonsynonymous (NS), Single nucleotide polymorphisms (SNPs)

TABLE A.7

COMPLETE LIST OF SNPS ANALYZED

GENETIC VARIATION IN ANTIOXIDANT ENZYMES AND LUNG

FUNCTION IN THE HEALTH, AGING, AND BODY COMPOSITION STUDY

Gene	Single Nucleotide Polymorphism	Minor Allele Frequency		Type
		European Americans	African Americans	
Catalase	rs564250	22%	20%	5' region
	rs769214	33%	41%	5' region
	rs484214	29%	29%	Intron
	rs7933285	27%	18%	Intron
	rs769217	23%	16%	Intron
	rs2420388	23%	18%	Intron
	rs475043	38%	10%	3' region
Glucose-6-Phosphate Dehydrogenase	rs2472394	10%	13%	Intron
Glutamate-cysteine ligase (catalytic subunit)	rs742528	26%	38%	3' region
	rs6458939	25%	42%	3' region
	rs7742367	19%	31%	3' region
	rs2066511	26%	25%	Intron
	rs16883894	9%	35%	Intron
	rs1555903	9%	27%	Intron
	rs622447	20%	17%	Intron
	rs600033	45%	36%	Intron
	rs4715407	8%	25%	Intron
	rs4712035	19%	34%	Intron
	rs2397147	37%	24%	Intron
	rs606548	6%	34%	Intron
	rs2284650	5%	6%	Intron
	rs510088	31%	18%	Intron
	rs17883901	8%	3%	5' region
Glutamate-cysteine ligase (modulatory subunit)	rs7549683	35%	36%	3' region
	rs769211	26%	24%	Intron
	rs7517826	35%	36%	Intron
	rs3827715	26%	25%	Intron
	rs2301022	31%	47%	Intron
	rs41303970	18%	19%	5' region
Gamma-glutamyl Transferase 1	rs16978740	3%	10%	Intron
	rs2154611	28%	32%	Intron
	rs6519519	26%	39%	Intron
Glutaredoxin	rs1047420	38%	15%	3' region
	rs4561	41%	32%	Synonymous
	rs3822751	25%	47%	Intron
	rs9314160	43%	42%	Intron
	rs3756704	47%	32%	5' region
Glutaredoxin 2	rs34552619	0%	8%	3' region
	rs35358794	1%	6%	Intron
	rs4657845	26%	25%	Intron
	rs10801174	32%	48%	Intron
	rs912071	27%	27%	5' region
	rs7547615	32%	48%	5' region
Glutathione Peroxidase 1	rs8179172	0%	9%	3' region
	rs3811699	33%	29%	5' region
	rs3448	25%	27%	5' region
Glutathione Peroxidase 2	rs10133054	22%	42%	3' region
	rs4902346	22%	41%	Intron
	rs2412065	22%	41%	Intron
	rs2737844	31%	21%	Intron
	rs17102360	7%	10%	5' region
Glutathione Peroxidase 3	rs1946234	14%	20%	5' region
	rs3828599	25%	43%	Intron
	rs8177435	39%	30%	Intron
	rs8177447	17%	33%	Intron

TABLE A.7 (continued)

Gene	Single Nucleotide Polymorphism	Minor Allele Frequency		Type
		European Americans	African Americans	
Glutathione Peroxidase 4	rs757228	45%	41%	5' region
	rs3746165	45%	42%	5' region
	rs4807542	18%	4%	Synonymous
	rs8178977	23%	30%	Intron
	rs2074451	50%	29%	3' region
Glutathione Peroxidase 7	rs6588431	39%	40%	5' region
	rs3753753	26%	13%	Intron
	rs946154	33%	41%	Intron
	rs1047635	45%	38%	3' region
	rs7529595	31%	12%	3' region
Glutathione Reductase	rs3594	39%	18%	3' region
	rs2250192	21%	35%	Intron
	rs8190996	43%	32%	Intron
	rs8190955	0%	8%	Nonsynonymous
	rs2978662	19%	34%	Intron
	rs2978296	19%	29%	Intron
	rs10088455	0%	35%	Intron
	rs8190907	0%	38%	Intron
	rs1002149	17%	24%	5' region
Glutathione Synthetase	rs725521	39%	46%	3' region
	rs6087651	39%	45%	Intron
	rs6087653	39%	46%	Intron
	rs2273684	45%	30%	Intron
	rs6060127	29%	20%	Intron
	rs6088659	19%	4%	Intron
	rs3761144	41%	24%	5' region
Glutathione S-Transferase A1	rs6920039	0%	9%	3' region
	rs6917325	44%	31%	Intron
	rs3756982	44%	31%	5' region
Glutathione S-Transferase A2	rs2180314	43%	33%	Nonsynonymous
	rs2180319	33%	7%	Intron
	rs2608629	37%	14%	Intron
Glutathione S-Transferase A3	rs10214816	3%	18%	3' region
	rs557135	37%	35%	Intron
	rs1052661	0%	13%	Nonsynonymous
	rs2281594	4%	40%	Intron
	rs614765	4%	43%	Intron
	rs563464	3%	33%	5' region
Glutathione S-Transferase A4	rs405729	45%	45%	3' region
	rs316133	39%	48%	Intron
	rs3756980	20%	11%	Intron
	rs6904771	2%	24%	Intron
	rs11967816	8%	7%	Intron
	rs13207376	8%	10%	Intron
Glutathione S-Transferase A5	rs4236107	0%	15%	3' region
	rs10948726	40%	9%	3' region
	rs7755335	4%	13%	Intron
	rs2397118	4%	13%	Nonsynonymous
	rs4715353	44%	38%	Intron
	rs4715354	46%	16%	Intron
	rs7749576	6%	42%	Intron
Glutathione S-Transferase K1	rs7803893	0%	4%	Intron
Glutathione S-Transferase M2	rs638820	48%	50%	5' region
	rs625456	14%	17%	Intron
	rs673151	6%	3%	Intron

TABLE A.7 (continued)

Gene	Single Nucleotide Polymorphism	Minor Allele Frequency		Type
		European Americans	African Americans	
Glutathione S-Transferase M3	rs1927328	31%	12%	3' region
	rs1537236	49%	41%	3' region
	rs7483	30%	12%	Nonsynonymous
	rs1571858	30%	15%	Intron
	rs10735234	43%	16%	Intron
	rs1332018	44%	19%	5' region
Glutathione S-Transferase M4	rs560018	36%	14%	Intron
	rs535537	13%	12%	Intron
	rs627365	14%	3%	Intron
	rs670439	14%	13%	Intron
Glutathione S-Transferase O1	rs2164624	34%	13%	5' region
	rs12259337	0%	25%	Intron
	rs17116741	0%	25%	Intron
	rs1147611	38%	28%	Intron
Glutathione S-Transferase O2	rs12264844	0%	25%	5' region
	rs7089730	0%	25%	Intron
	rs7070750	0%	34%	Intron
	rs157077	46%	25%	Intron
	rs156697	35%	29%	Nonsynonymous
	rs276203	35%	29%	Intron
	rs157076	32%	33%	Intron
	rs157080	32%	31%	Intron
	rs3740466	29%	24%	3' region
Glutathione S-Transferase P1	rs6591256	39%	38%	5' region
	rs4147581	49%	16%	Intron
	rs8191446	0%	12%	Intron
	rs1695	32%	45%	Nonsynonymous
	rs749174	33%	26%	Intron
	rs743679	0%	12%	Intron
	rs1138272	8%	1%	Nonsynonymous
	rs947895	33%	26%	3' region
Glutathione S-Transferase Z1	rs2111699	32%	31%	Intron
	rs2363643	32%	31%	Splice Site Donor
	rs2270422	39%	15%	Intron
	rs3177427	34%	29%	Nonsynonymous
	rs1046428	20%	9%	Nonsynonymous
	rs1017186	20%	11%	3' region
Heme-Oxygenase 1	rs6518952	0%	32%	Intron
	rs2071749	46%	12%	Intron
	rs5755720	35%	20%	Intron
	rs2285112	41%	34%	Intron
	rs17883419	6%	11%	3' region
Heme-Oxygenase 2	rs11639998	26%	22%	Intron
	rs8055559	4%	26%	Intron
	rs11643057	28%	35%	Intron
	rs1362626	27%	48%	Intron
	rs2270366	32%	25%	Intron
	rs1051308	31%	25%	3' region
Isocitrate Dehydrogenase 1	rs7593466	21%	35%	3' region
	rs7565247	8%	42%	Intron
	rs34218846	7%	8%	Nonsynonymous
	rs6435435	7%	13%	Intron
	rs12612631	0%	14%	Intron
	rs1437410	42%	22%	Intron

TABLE A.7 (continued)

Gene	Single Nucleotide Polymorphism	Minor Allele Frequency		Type
		European Americans	African Americans	
	rs10207062	42%	22%	5' region
Isocitrate Dehydrogenase 2	rs7176347	0%	12%	3' region
	rs9972549	2%	43%	Intron
	rs11073899	17%	20%	Intron
	rs8030346	41%	28%	Intron
	rs8028234	25%	35%	Intron
	rs4932279	44%	24%	Intron
	rs7182369	25%	34%	Intron
Isocitrate Dehydrogenase 3A	rs11631100	42%	47%	Intron
	rs8032618	50%	33%	Intron
	rs7180781	1%	11%	Intron
	rs3816253	44%	48%	Intron
	rs17674205	8%	2%	3' region
Isocitrate Dehydrogenase 3B	rs6037255	26%	41%	Intron
	rs6115381	7%	37%	Intron
	rs6107100	7%	37%	Intron
	rs2073192	7%	5%	5' region
Isocitrate Dehydrogenase 3G	rs2071122	28%	35%	Intron
	rs2071123	28%	34%	Intron
	rs2071124	7%	10%	Intron
	rs17429	0%	22%	Intron
	rs2283753	7%	10%	Intron
	rs4898445	35%	26%	Intron
Microsomal Glutathione	rs7970208	47%	29%	5' region
S-Transferase 1	rs7294985	24%	36%	Intron
	rs2975149	25%	42%	Intron
	rs2075237	20%	15%	Intron
	rs4149197	31%	46%	Intron
	rs9332939	50%	31%	Intron
	rs3852576	49%	22%	Intron
	rs7135371	49%	31%	3' region
Microsomal Glutathione	rs8191997	16%	26%	5' region
S-Transferase 2	rs8192004	9%	6%	5' region
	rs1000222	39%	48%	Intron
	rs795594	47%	23%	Intron
	rs8192047	26%	9%	Intron
	rs795590	2%	9%	Intron
	rs795589	42%	49%	Intron
	rs8192066	0%	7%	Intron
	rs7664313	21%	39%	Intron
	rs2646076	22%	28%	Intron
	rs2646035	17%	42%	3' region
Microsomal Glutathione	rs7549530	16%	19%	5' region
S-Transferase 3	rs10737515	47%	20%	5' region
	rs9333378	38%	36%	Intron
	rs10800120	40%	20%	Intron
	rs7554034	21%	46%	Intron
	rs957644	26%	46%	Intron
	rs9333471	29%	44%	Intron
	rs4147602	31%	43%	Intron
	rs2297765	44%	36%	Intron
	rs7533986	30%	26%	Intron
	rs10494446	14%	29%	Intron
Peroxiredoxin 1	rs4660306	35%	19%	Intron
	rs2152077	35%	41%	Intron

TABLE A.7 (continued)

Gene	Single Nucleotide Polymorphism	Minor Allele Frequency		Type
		European Americans	African Americans	
	rs713358	22%	25%	5' region
Peroxiredoxin 2	rs10404253	1%	23%	3' region
	rs35866106	1%	5%	Intron
	rs10413408	3%	18%	Intron
	rs1205171	18%	5%	Intron
	rs10422248	3%	16%	Intron
	rs8107906	3%	18%	5' region
Peroxiredoxin 3	rs7068937	47%	19%	3' region
	rs7768	33%	44%	3' region
	rs11198808	13%	16%	Intron
	rs4752257	13%	19%	Intron
	rs11198811	32%	48%	Intron
	rs1553850	42%	23%	5' region
Peroxiredoxin 4	rs557914	40%	37%	Intron
	rs513573	40%	38%	Intron
	rs528960	9%	24%	Intron
	rs518329	40%	38%	Intron
	rs795489	42%	32%	Intron
	rs1548734	40%	38%	Intron
	rs477233	42%	41%	3' region
Peroxiredoxin 5	rs9787810	32%	7%	5' region
	rs7938623	0%	19%	Nonsynonymous
	rs7941847	0%	12%	Intron
	rs566049	0%	13%	Intron
	rs1047206	15%	5%	3' region
	rs4930702	14%	25%	3' region
Peroxiredoxin 6	rs34619706	10%	2%	5' region
	rs34977864	0%	7%	5' region
	rs7540065	25%	38%	Intron
	rs9425725	0%	20%	Intron
	rs34308867	0%	10%	Intron
	rs6702835	24%	41%	3' region
Selenoprotein P 1	rs230813	46%	45%	3' region
	rs6413428	25%	34%	3' region
	rs3877899	24%	27%	Nonsynonymous
	rs1046068	29%	15%	Intron
	rs230820	45%	45%	Intron
	rs6865453	26%	7%	Intron
Selenoprotein W 1	rs11670990	33%	43%	5' region
	rs1862485	31%	25%	Intron
	rs3815751	33%	46%	Intron
	rs10412896	36%	42%	Intron
	rs10427074	0%	12%	Intron
	rs3786777	48%	11%	Intron
	rs2042286	42%	42%	3' region
Superoxide Dismutase 1	rs11910115	0%	12%	5' region
	rs4998557	11%	39%	Intron
	rs2070424	6%	19%	Intron
	rs1041740	32%	10%	Intron
Superoxide Dismutase 2	rs4342445	22%	15%	3' region
	rs2758329	50%	41%	3' region
	rs8031	49%	34%	Intron
	rs2758331	49%	25%	Intron
	rs4880	48%	44%	Nonsynonymous
	rs2758346	49%	44%	5' region

TABLE A.7 (continued)

Gene	Single Nucleotide Polymorphism	Minor Allele Frequency		Type
		European Americans	African Americans	
Superoxide Dismutase 3	rs2284659	37%	18%	5' region
	rs8192287	7%	2%	5' region
	rs699474	2%	9%	Intron
	rs17885542	1%	13%	Intron
	rs17878863	8%	1%	Intron
	rs7655372	1%	8%	Intron
	rs1007991	34%	22%	Intron
	rs1799895	1%	0%	Nonsynonymous
	rs2855262	37%	18%	3' region
	rs2695234	9%	50%	3' region
Thioredoxin 1	rs2026312	42%	35%	3' region
	rs1964889	34%	25%	Intron
	rs2418076	27%	44%	Intron
	rs4135191	2%	10%	Intron
	rs4135168	25%	28%	Intron
Thioredoxin 2	rs5995291	11%	34%	Intron
	rs8139906	18%	40%	Intron
	rs2283965	19%	24%	Intron
	rs2267337	19%	22%	Intron
	rs2281082	20%	22%	Intron
Thioredoxin Reductase 1	rs11111979	46%	32%	5' region
	rs4523760	24%	26%	Intron
	rs5018287	46%	37%	Intron
	rs4595619	46%	32%	Intron
	rs17202060	34%	17%	Intron
	rs7138318	32%	39%	Intron
	rs11610799	8%	13%	Intron
	rs4964785	46%	38%	3' region
Thioredoxin Reductase 2	rs7285948	16%	38%	3' region
	rs1139795	17%	45%	Synonymous
	rs1139793	26%	10%	Nonsynonymous
	rs2073750	23%	44%	Intron
	rs9606173	15%	18%	Intron
	rs5992493	18%	50%	Intron
	rs3788314	48%	28%	Intron
	rs756661	44%	25%	Intron
	rs5748469	36%	24%	Nonsynonymous
	rs5748471	44%	23%	Intron
	rs9306229	22%	15%	Intron
	rs737866	29%	14%	5' region
Thioredoxin Reductase 3	rs9834240	35%	38%	Intron
	rs1520850	34%	46%	Intron
	rs777238	12%	50%	Intron
	rs9637365	42%	27%	Intron

TABLE A.8

Most statistically significant SNPs in models with small numbers in genotype/smoking subgroups[a]

Population	Outcome	SNP*smoking parameter[b]	Gene	Function	P-value[c]
European	$ppFEV_1$	rs38778993*Status	*SEPP1*	Selenoprotein	0.001
Americans		Rs34619706*Dose	*PRDX6*	Peroxidase Activity	0.001
	FEV_1/FVC	Rs2154611*Status[d]	*GGT1*	GSH Synthesis	0.001
		Rs6519519*Status[d]	*GGT1*	GSH Synthesis	0.001
		Rs8177447*Status	*GPX3*	Peroxidase Activity	0.002
		Rs2154611*Dose[d]	*GGT1*	GSH Synthesis	0.0002
		Rs6519519*Dose[d]	*GGT1*	GSH Synthesis	0.0002
		Rs17102360*Dose	*GPX2*	Peroxidase Activity	0.002
		Rs8177447*Dose	*GPX3*	Peroxidase Activity	0.0002
		Rs7749576*Dose	*GSTA5*	Glutathione S-Transferase	0.0005
		Rs1046428*Dose[e]	*GSTZ1*	Glutathione S-Transferase	0.0002
		Rs1017186*Dose[e]	*GSTZ1*	Glutathione S-Transferase	0.0002
		Rs795590*Dose	*mGST2*	Glutathione S-Transferase	0.002
		Rs4342445*Dose	*SOD2*	Superoxide Dismutase	0.002
African	$ppFEV_1$	Rs699474*Dose	*SOD3*	Superoxide Dismutase	0.002
Americans	FEV_1/FVC	**Rs2284650*Status**	*GCLC*	Glutathione Synthesis	0.000001
		Rs2071749*Status	*HMOX1*	Heme-Oxygenase	0.0004
		Rs3786777*Status	*SEPW1*	Selenoprotein	0.0006
		Rs528960*Status	*PRDX4*	Peroxidase Activity	0.002
		Rs2042286*Status	*SEPW1*	Selenoprotein	0.0002
		Rs2284650*Dose	*GCLC*	Glutathione Synthesis	0.0000008
		Rs2978296*Dose	*GSR*	Disulfide Reductase	0.001
		Rs563464*Dose	*GSTA3*	Glutathione S-Transferase	0.00001
		Rs2071749*Dose	*HMOX1*	Heme-Oxygenase	0.001
		Rs7176347*Dose	*IDH2*	Reducing Equivalents	0.0009

[a]All results with nominal p-value <0.002

[b]SNPs (single nucleotide polymorphisms) in **bold** remained statistically significant after adjustment for multiple comparisons

[c]Nominal p-values shown

[d]Strong LD observed between these SNPs (R^2=0.93)

[e]Strong LD observed between these SNPs (R^2=0.90)